

An Original Thesis

On a New Method of War-Wound Treatment
by the Introduction to the Wound
of Living Cultures of a Spore-bearing Anaerobe
of the Proteolytic Group
Embracing
a Description of the Morphological and Cultural
Characters of the Organism
together with certain Experimental Investigations
directed towards determining its possible Modus
operandi
(illustrated)

by

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P R E F A C E.

In the accompanying thesis, I have endeavoured to give an account of the bacteriological and biological characters of an organism - the Reading bacillus - which I have isolated from war wounds, of the relationship of the organism to such wounds, and of its probable mode of action.

Based on a study of the facts elucidated in this way, I have elaborated an entirely new method of treatment for gun-shot wounds in which the Reading bacillus plays the chief part. This I have called the Biological Method to distinguish it from those known as the antiseptic, the physiological and the surgical respectively, while at the same time it signifies that the chief agency responsible for cleaning these wounds is a living one. So far as I am aware, no such method has ever before been advocated, and its successful employment therefore constitutes a complete break with tradition.

I am aware that to advocate the introduction into infected gun-shot wounds, of living cultures of a bacillus which, while harmless in itself, belongs to a class which embraces organisms capable of causing deadly symptoms, is a daring thing to do.

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It has now, however, been done not once only but many times, until it has become a routine procedure in the wards of the Reading War Hospitals.

This thesis embodies the reasons, such as I have to offer, for the faith I have in this new biological method and it deals with the attitude I take up in relation to war wounds, more especially with the significance to be attached to the presence of dead tissue in such wounds. It is at once a plea for a more scientific appreciation of the morbid processes at work and for a more rational form of treatment founded thereon.

The thesis and the work on which it is based are my own with the exception of one or two of the purely clinical observations, made by my colleague Major Joyce, R.A.M.C.T., and acknowledged where they occur in the text. It was his initial observation that some cases emitted a certain odour while others did not, that led to my entering on this research.

While the theories built up in this thesis and founded on my own clinical observation and laboratory research, are entirely my own, I am aware that they are shared, in all essential points so far as their clinical application goes, by those of my colleagues/

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colleagues who have had most opportunity to follow the progress of the treatment. Before going further I wish to acknowledge, in the first place, the kindness of Majors Joyce, Price and Guilding, Captain Foster, Dr. Franklin Cox and Dr. Norman Joy, in granting me access to their patients, in according me every facility for the collection of material, and for placing all clinical records at my disposal.

I wish further to express my indebtedness to Mr. R. C. McLean, D.Sc., and to Miss A. B. Clark, B.Sc., my war-time assistants whom I have had the pleasure of initiating into bacteriological methods. They have never grudged to render me every assistance in their power, and by relieving me of much of the routine laboratory work, have enabled me to devote more time to this research than would otherwise have been possible.

In this connection I wish to acknowledge the willing services of my laboratory attendants. I am also indebted to Miss Muriel Robertson, of the Lister Institute, London, and to Dr. MacConkey, Superintendent of the Serological Department, Lister Institute, Elstree; to the former for strains of bacillus perfringens and of vibron septique; to the latter/

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latter for supplying me with the fluid toxins of tetanus and diphtheria required for certain experimental work in connection with this research.

Lastly, I must express my indebtedness to Dr. O'Brien, Superintendent of the Wellcome Physiological Research Laboratories, London, who kindly supplied me with dessicated tetanus toxin, and to J. E. G. Harris, B.Sc., Sergeant R.A.M.C.T. attached to a Base Hygiene Laboratory in France, for kindly furnishing me with cultures of bacillus sporogenes (Metchnikoff) and bacillus histolyticus for purposes of comparison, with the Reading bacillus.

A New Method of War Wound Treatment—Biological.
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The efficient treatment of wounds is one of the oldest medical problems with which man has been confronted, and is one which still awaits solution. From age to age an answer has been attempted consciously or unconsciously by the various tribes and races that have passed through the world, but while the methods of treatment adopted have been various, they all agree, so far as we know, in one respect, viz: that they were based on empiricism. They were the result of natural selection, and while each may have had some value under certain conditions of life, that value gradually diminished with the advent of modern civilisation. How bad these methods had become can be read in the published accounts of wound treatment as late as the days of Lister. Indeed, there are those still living who can recall their personal experience of pre-Listerian methods. To us who belong to a younger school this seems a fact difficult to realise in view of the immense strides which Science has made in the course of a comparatively few years. The discoveries of Pasteur and the work of Lister initiated a new era in medicine, and opened up a great new field for scientific exploration.

To the former we owe our knowledge of the reason why/

why wounds go wrong - the significance of organismal implantation, and to the latter our knowledge of how this may be prevented.

Lister's work lay in the direction of prophylaxis - prevention of the entry of organisms by the use of antiseptics. From this it was but a step to the use of antiseptics in wounds which had already become infected, and in this way the era of antisepsis was ushered in.

Wounds came, however, to be differentiated into two categories, those made by the surgeon into non-infected tissues, and those inflicted by other means and into which pathogenic germs had gained a footing. With a recognition of this distinction came a slight modification of Lister's methods. It was argued that since a wound made by the surgeon into non-infected tissues contains no organisms, it was not necessary to introduce into such wounds any antiseptic, especially as the latter often possessed irritating properties which militated against rapid healing. It was sufficient if the skin only were rendered more or less sterile by the use of an antiseptic, while the instruments, etc. were freed from organisms by boiling.

In this way the aseptic method of treatment came into being, for such wounds as were inflicted by the surgeon on clean tissues.

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In the use and application of these two methods the younger generation of medical men has been trained, and in view of the facilities existing in civil life for the rapid and thorough treatment of freshly infected wounds, these methods have been found on the whole satisfactory in the majority of cases. With various adjuncts such as vaccine therapy, wound infections had largely been robbed of their terrors. With the outbreak of the present great war, however, this false sense of security was shattered. Like a bolt from the blue it was found that the old antiseptics were comparatively powerless to stay the ravages made by infecting organisms. Men were unaccustomed to deal with such wounds or with such heavy infections. Apart from the extensive destruction and laceration of the tissues, their mode of infliction by its very nature carried infection deeply into the wound. Moreover, the infecting flora were of such variety and virulence as had hitherto been unknown in the course of ordinary civil practice. It was easy after the event to understand that it should be so when we consider how very highly manured is the soil on which the fighting is taking place. Further, the conditions of trench warfare which evolved after the preliminary manoeuvring of the hostile armies, were such as literally to saturate the clothing and to plaster the bodies of/

of the soldiers with mud and filth highly charged with faecal organisms. The character of the wounds caused by modern weapons of destruction and the unusually heavy contamination of these wounds were factors entirely new to men who had had to deal only with the wounds and infections of civil life.

It was natural that medical officers should have recourse to the strongest weapons they knew for combating infection, but it was speedily found that in many cases these weapons, the usual antiseptics, were powerless in their hands.

It was at this juncture that Sir Almroth Wright¹⁾ came forward as the opponent of antiseptic treatment, and the apostle of his so-called "physiological treatment" by means of hypertonic saline solutions.

The object of this thesis, however, is not to discuss the question of antiseptics versus hypertonic saline, nor is it my intention to canvass the theories or criticise at any length the methods of treatment advocated by Sir Almroth Wright. The theories of the latter have already been subjected by other competent workers to trenchant criticism. Moreover, that Wright himself has not only modified his original methods of application of the hypertonic solution, but has even modified his original views as to its mode of action, seems to point to the fact that a complete understanding of/
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of the physics and of the biological properties of hypertonic saline has yet to be achieved.

From the practical point of view, while it might only show ignorance on their part or inability to use this method of treatment as Wright suggested, the fact that it has been abandoned by many for other methods seems significant. The chief merit of Wright's work seems, in my opinion, to lie in the fact that he helped to break the spell which had hitherto bound surgeons to the use of antiseptics.

Hitherto, in regarding wound treatment, the surgeon had eyes only for the infecting organisms. The septic wound was to him a solution of the continuity of some part of the body into which organisms had gained an entrance. The only factor, or at least the chief factor which rendered the wound unhealthy, which prevented its healing, and which in certain cases even menaced the patient's life, were the infecting organisms. Obsessed with this idea, it was his chief aim to rid the wound of its infecting flora, and for that purpose until recently the chief weapon in his armamentarium was the antiseptic. As I have said, however, this weapon failed him when he was brought face to face with the wound infections of modern warfare as existing at present in Europe. Wright came forward at this point and directed the attention of the surgeon to a second/

second factor in wound treatment, the importance of which the latter had not sufficiently recognised, viz: the protective mechanism of the patient's own tissues. Extraneous attack on the organisms by antiseptics was not the only way of fighting infection. The patient possessed a protective mechanism which ought to be given full scope for action and according to Wright this was best obtained by abstaining from the use of antiseptics and by employing hypertonic solutions of salt. The fact that he laid emphasis on the ability of the patient to combat his own infection, if given a chance, seems to me of more importance than the particular method which he devised to attain this end.

To condemn the use of antiseptics indiscriminately was in my opinion wrong, but it was perhaps natural, when for the time being they were found to be impotent in so many cases. It was natural that the pendulum should swing in the opposite direction, and that antiseptics, hitherto implicitly relied upon, should now come to be regarded as wholly useless. Nay, it may even be that Wright's dogmatic statements expressed in no uncertain terms, were necessary in order to bring home to the surgical mind the fact that the patient's own body cells possessed a natural power of defence, and that the surgeon's antiseptic was not the only nor even the main weapon against invasion by hostile microbes./

microbes.

In this way, accordingly, there came to be in use two main lines of treatment, both directed towards ridding the wound of infection, but diametrically opposed in method. So far as this conception of wound infections goes, there is something to be said for both views. If we consider the antiseptic school, we find that its adherents are far from being in agreement as to the best antiseptic to employ. The materials used as antiseptics cover a wide range, and have been used in many different combinations. In the hands of one man better results are got with a certain antiseptic, for example, phenol or iodine, than with any other. Another will wholeheartedly condemn that antiseptic as utterly useless, and in its stead will advocate something totally different in nature. Each different antiseptic has its devotee. The truth probably consists in the fact that one particular antiseptic may be useful in the treatment of one type of wound, while in another it may be of little or no value whatever. Further, the application of one method may be good in one part of a given wound, while in another part an entirely different treatment should be practised, and finally, what may be good at one period of the wound's history may be bad at another. Because it has been useful in one type of wound, it does/

does not follow that such an antiseptic is of universal utility or even that it is of equal value during the whole period that is necessary for repair.

Abundant examples might be given to illustrate these points, but as this thesis is not directly concerned with a full discussion of the merits and demerits of antiseptics, it is not necessary to elaborate the point further.

I hold no brief either for the antiseptic or for the physiological schools of treatment. I merely wish to indicate that the former do not deserve to be utterly condemned by the latter. The multiplicity of antiseptics employed by adherents of the first named school, points not to the fact that all such antiseptics are bad, but rather to the fact that each particular wound, if it is proposed to treat such with an antiseptic, must be studied independently, and that chosen which will be most suitable. But it points further to another consideration, viz: that a septic wound consists not merely of a solution of continuity of some part of the body to which organisms have gained an entry. The physiological school, while rightly insisting on the fact that the body should be allowed the fullest scope for staying the progress of infection and for effecting repair, are not only wrong in condemning the use of antiseptics because their use is not/

not invariably followed by improvement in the condition of the wound, but they fall into the same error as do the adherents of the antiseptic school. They both regard wounds much in the same way, viz: as a solution of body continuity with an organismal implantation. They differ, however, in the precise importance which they attach to these two factors. In the case of the first named, the defensive mechanism of the body ought to be the chief factor to be turned to account in combating the infection. In the case of the second-named school, while this defensive mechanism receives scant attention, their main effort is concentrated on the attack upon the organisms from the outside by chemical means. The latter think largely in terms of antiseptic and organism, due in part to the fact that in many cases their arguments are based on purely "in vitro" investigations of the antiseptic employed. They are both obsessed with the idea that the organism is the main factor in a war-wound infection, but while one school think only of how they can exterminate the infection by artificial means and neglect the assistance that may be obtained from the patient's own body, the other school think only of utilising to the full the patient's own defensive mechanism, while they omit to use any measures in the shape of the ordinary antiseptics/

antiseptics in common use.

They are probably however both in error, in so far as their conception of an infected war-wound does not take into consideration all that is involved in such a wound, or at any rate does not attach to each factor its due importance. An infected war-wound does not merely consist of a solution of continuity of the body into which organisms have been implanted.

There is a third factor and one to which sufficient importance has not hitherto been attached, viz: the presence in that wound of devitalised or dead tissues. The missile which inflicts the trauma does not merely cause a solution of the continuity of some part of the body, it does not merely carry in pathogenic organisms, but it devitalises more or less of the living tissues, and it is the importance of this last factor that has been largely lost sight of. That it has forced itself on our attention is evident from the fact that many now advocate complete and immediate excision of a wound. As I have already indicated, at the beginning of the present war antiseptic treatment predominated. For reasons which I have hinted at, this form of treatment yielded ground to the newer physiological methods recommended so persistently by Wright. These in turn, however, have been abandoned by many who have given them/

them fair trial in favour of more radical treatment by the method of complete excision. In other words, while success is achieved in the treatment of certain wounds treated in some cases on antiseptic principles, in others on physiological, yet neither is of universal application, neither can be relied on a priori to effect a cure, and both are liable to fail with disastrous consequences to the patient. There must be some explanation probably common to both for the failures which are constantly being encountered by either of these methods, and this explanation I venture to submit will be found when we understand clearly the rôle played by dead and devitalised tissues in a wound, and when we understand the biological processes involved in the relationship of implanted bacteria to the damaged tissue. The fact that many of the most eminent surgeons now recommend and practise complete excision of war-wounds where such a proceeding is feasible, not only clearly indicates a recognition of the fact that neither the antiseptic nor the physiological method of treatment can be implicitly relied upon for success, but also implies that they have at length begun to realise the importance of bestowing attention on the damaged tissues. I would submit that the time has come for a revision of the surgical definition of a wound/

wound as "a solution of continuity in any part of the body suddenly made by anything that cuts or tears, with division of the skin." I would venture to suggest that in that definition be incorporated due reference to the fact that it is not only a solution of the continuity of some part of the body, but a solution accompanied by the devitalisation or even the death of some part of the tissues involved. Such a definition would bring home to the student the fact that even a simple surgical incision does not merely mean a solution of the continuity of some part of the body, however small, but that it also means partial and localised death. When this truth has been fully realised, the sequelae of wound infliction will be understood. From the wound inflicted by the surgeon's knife, in which the devitalised tissue is small in amount and from which organismal infection has been rigidly excluded, will be all gradations of severity of wound infection depending mainly on the amount of dead tissue present, and the opportunities this gives for organismal growth and activity if bacteria have become implanted.

I have laboured this point somewhat, for it seems to me that on a recognition of these facts depends to a certain extent the solution of certain problems on which this thesis is based.

I have endeavoured to show, in this survey of wound treatment, that three main lines of attack have been followed during the present war, viz: the "anti-septic" governed by the idea that the infecting bacteria could be exterminated or kept in subjection by the application, from the outside, of chemicals found in vitro to be able to kill bacteria when applied in a given strength and for a given time; the "physiological," where the living body cells were encouraged to deal with the invading organisms by biological methods and the "surgical," by which I mean the removal at the earliest possible moment of the damaged tissues by the surgeon's knife.

I wish now to go back to the second of these, viz: the physiological, devised by Sir Almroth Wright. I have already stated what, in my opinion, is the chief value of his work, with an analysis of which this thesis is not concerned. The means, however, by which he sought to achieve the end he had in view, was indirectly the stimulus which prompted the investigation, the details of which form the present thesis. Wright called attention to ordinary salt as the means par excellence of inducing the body to undertake its own defence against invading bacteria. Out of the method advocated by him in this way another was evolved, and this offshoot of Wright's technique in turn gave birth to/

to the new method which forms the main argument of my thesis.

Col. C. B. Lawson and Col. H. M. W. Gray, C.B.,²⁾ basing their theories of treatment on those formulated by Wright, introduced, in order to promote a so-called lymphagogue action and to obviate the need for elaborate drainage or continuous irrigation, the method of treatment by "salt packs".

Briefly put, the merit of the salt pack lies in the ease with which it can be applied, in the fact that it can be left in situ for 5 or 6 days without requiring renewal with great comfort and advantage to the patient, and in the fact, according to its inventors, that it effects more or less closely the changes which Wright insists upon are necessary for the rapid and successful cleansing of a wound from infection. Whether or not the salt acts physically, as Wright and his followers believe, I do not propose at this stage to discuss, although on the lines of their explanation there is much that requires further elucidation. The physics, not to talk of the biological processes involved in its use, require considerably more in the nature of accurate scientific explanation than has been advanced.

Suffice it to say, that according to them, the salt acts in the first instance as a lymphagogue, so preventing/

preventing a wound becoming what Wright terms "lymph-bound", that the lymph flow tends to loosen sloughs so that they come away more readily, that later the salt solution of reduced tonicity has a chemio-tactic influence on the leucocytes which together with the salt present complete the victory over the invading bacteria. In addition to the active defence in the form of phagocytes and what he calls "bacteriotropic" substances,³⁾ there is the passive defence which he defines as "the protection against infection obtained by preventing microbes converting to their uses the nutrient substances of the blood fluids." In other words the mastery over infection will be obtained in part by the raising of the anti-tryptic power of the blood, which he says is increased in all severe wound infections.

Now, as pathologist in charge of the Laboratories of the Reading War Hospitals, it fell to my lot, amongst other things, to investigate specimens of pus from a very large number of wounds. These wounds were being treated in all manner of ways according to the caprice or preference of the surgeon who happened to be in charge of them. Of the surgeons one, Major (then Capt.) J. L. Joyce, R.A.M.C.T. early became a convert to the salt pack method of treatment with results which were in the majority of cases all that could be desired, and which coincide with the published results/

results obtained by all those who have given it a fair trial. The adoption of this method has considerably curtailed the patient's period of convalescence, and has, moreover, succeeded where other lines of treatment have failed, not excluding the much-advocated and much belauded Carrel-Dakin procedure.

It was my good fortune to find amongst the surgical staff one who took a keen and intelligent interest in the clinical course of the wound infections under his charge and facilities were given me for investigation of those wounds in a way that was impossible in the case of wounds under the other surgeons who followed no consistent method of treatment.

The wounds were generally at least 5 or 7 days old before the patients arrived in Reading, and they came with a variety of dressing. They were mostly gun-shot wounds, and, as such, practically always involved muscle, parts of which were dead or dying as a result of the trauma. The tissues were generally dirty, sloughy and purulent, while the surrounding areas were frequently oedematous, and almost always inflamed. Where the patient was obviously ill, in pain, and running a temperature, it was marvellous to find how in three days, as a rule, after the salt pack had been introduced, the temperature would fall to normal, and how in a week or ten days' time, the wound would be
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a healthy, bright red colour, well covered with firm granulations. This result was achieved with the minimum of discomfort to the patient, since, after the first packing, it was unnecessary to redress it for some 5, 6 or 7 days. In this way rest, one of the great allies in successful healing, was obtained. The fall in temperature and the mental quietude engendered by the knowledge that no daily dressing was to be looked forward to, contributed to the patient's well-being. He was able to obtain refreshing and normal sleep, and his appetite generally improved, so that many of the vicious circles in operation were thus broken.

Impressed with the results obtained by this method of treatment, the question naturally arose, in what way had they been brought about? Was the bacterial content of wounds so treated actually less than before the commencement of the treatment? In other words, apart from the claims of the protagonist of the method as to its physical and biological properties in the wound, had the salt any definite or marked bactericidal action either directly on the organisms or indirectly through the leucocytes? To this end I made a series of observations before the introduction of the salt pack and again after its removal. The results were disappointing. The bacterial flora seemed to be as/

as numerous immediately after removal of the salt pack as before its use, and the investigation was abandoned, partly as unlikely to throw much light on the action of the salt pack and partly owing to pressure of routine work at the time. The method employed was to take a sample of the pus and, after diluting it, to plate out a fraction on each of four plates, two of which were incubated aerobically and two anaerobically. This was done with each sample, the resulting number of colonies was counted and the various types of organism noted. As these comparative results do not materially concern the main line of argument in this thesis, I shall omit all further reference to them. Suffice it to say that, in spite of the persistence of pathogenic organisms in apparently undiminished numbers, the results of the salt bag treatment quâ the healing of the wound were excellent, but why they should be so, remained as much a mystery as before.

That the wounds so treated did well and got well probably sooner and with less disturbance than by any other method was a clinical fact beyond dispute. This experience coincides exactly with the published statements of all those who have employed the salt pack method of treatment. Girling Ball, for example, states that "the salt causes an exudation of fluid "which washes out the bacteria not only from the "surface/

"surface of the wound but also from the deeper tissues,
 "thus affecting them in a manner which no antiseptic
 "applied to the surface will do. Whether this is due
 "to osmosis or irritation it is difficult to say; the
 "clinical fact remains it is a great advance
 "in the treatment of infected wounds."

Girling Ball,⁴⁾ impressed with the results he has obtained, is unable to explain how the results have been achieved although he offers a tentative suggestion. That this latter is probably incorrect is suggested by the fact that the outflow of fluid which occurs after the salt bags have been inserted, takes place for the most part entirely within the first twenty-four hours after insertion, and by the fact that the bacterial content may be as great after as before application of the bags. He is evidently still under the influence of Wright's teachings.

Roberts and Statham⁵⁾ declare that cases received from the clearing stations treated by the salt bag method arrive in excellent condition, much better than those treated by other methods. Their advocacy of the treatment, they say, is based entirely on clinical grounds, and they make no attempt to advance a hypothesis by way of explanation.

Similarly others who have described their experiences with salt bags in the treatment of wounds agree in/

in saying that while from the clinical standpoint, the results are excellent, they are unable to furnish any explanation of the principles governing its action, or if they do attempt a hypothesis, it is generally coloured by Wright's theories.

With this same problem still unsolved, it was noticed by my surgical colleague Captain Joyce, that while the majority of cases treated with salt bags did well and followed a more or less stereotyped course towards complete recovery, one or two cases were outstanding exceptions, and recourse was had in these to other methods of treatment. Why some cases, treated in exactly the same way as the others, should not improve, formed a new problem, until a clinical observation - and on this hinges the subsequent work which forms the main part of this thesis - was made by Captain Joyce. It had been noted by all who came in contact with wounds so treated, as it has been noted by all who have adopted this particular method, that a powerful and most offensive odour was emitted from the salt packed wounds. Now while septic wounds emit various odours, we have always regarded such as something to be avoided or to be got rid of when present. In some undefined way they have come to be regarded as correlating an unhealthy condition of the wound. Yet in all the cases successfully treated by the salt bag method/

method the particular offensive odour characteristic of these wounds has been invariably present, till now it has come to be regarded in Reading as a sign that all is going well with the wound. It is interesting to note how this odour, remarked upon by all who have used the salt pack method of treatment, is regarded by them. Most of them, like Girling Ball, have come to the conclusion that it is not to be looked upon as an indication for changing the salt pack. In other words, its existence, although unpleasant, is simply to be disregarded. Others with less fortitude lay it down as an axiom that when the odour begins to make itself felt, it is time to change the dressings!

None of them attempts an explanation except one ^{6.)} who puts forward the view that it is due to a "decomposition in the dressings themselves", a view that tells us little or nothing.

It was noted, however, by my colleague in the course of his clinical work, that one or two of those cases which failed to improve after the introduction of salt bags, were also devoid of this peculiarly offensive odour, and, on recalling to mind one or two previous cases that had not progressed satisfactorily in the usual way, it was remembered that they too had been devoid of this odour.

Having made this observation, my colleague came to/
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to me and propounded the riddle, Why do the cases which do well smell, while those which do not improve are also devoid of the characteristic odour? My answer was that there was probably a germ present in the first type of wound that was absent in the second.

The idea was conceived that the salt bag might possibly produce physical conditions in the wound which favoured the growth of a certain organism, and that consequently the good results of the treatment might in some way be directly or indirectly due to its activity. What the favouring physical conditions might be I had no idea, except that it was in some way connected with this particular method of salt application. Judging from the odour with which I was familiar, I considered that most likely the organism to search for would be an anaerobe, and as the point raised was one of some interest I resolved to put the matter to the test. A case was chosen from which the salt bag was due to be removed. This case was one which had obviously done well under the salt bag treatment, and which certainly emitted a most offensive and characteristic odour. From this case I collected some of the pus and sowed it in tubes containing minced up bullock's heart and broth made according to the method described by Miss Muriel Robertson.⁷⁾ After two or three days' anaerobic/

anaerobic incubation at 37° Cent. films were made in which I found, amongst other things, two types of anaerobic spore-bearing bacilli. One type was a slender rod possessing a round terminal spore and present in very small numbers. The other was a stouter rod with a large subterminal oval spore. The latter type was present in large excess over the round spored bacilli.

The meat broth tubes in which the pus had been incubated obviously contained a mixture, and as they showed evidence of active proteolysis and emitted the same powerful and offensive odour as the wounds did, I was inclined to think that the oval spore-bearing anaerobe, as it was present in predominant numbers, was responsible. If this supposition were correct, I felt that the presence of this organism, if it could be proved to be present in all wounds doing well under the salt bag treatment, would go a long way to explain the good results obtained in cases so treated. My first business was to obtain the organism in pure culture, and in view of the revelations made by Miss Robertson, I recognised that it would be no easy matter. It was simple to get rid of the non-spore-bearers by heating, but I was left with two anaerobic spore-bearers to be separated. At first I tried repeated plating and the transference of one colony or part of a colony from one/

one plate to the other, but was unsuccessful. I then hit upon the following method, which I have already described in one of the Journals.^(8.) The ideal method, of course, would be to raise a strain from a single cell - a method at the best of times tedious and difficult, but at the present time wholly impossible with so much urgent routine war work to be done.

Author's Method of obtaining Anaerobic Spore-Bearers in Pure Culture.

An isolated colony or part of such a colony is picked off from the solid medium with a platinum needle, and a suspension of this material is made in one or two c.c. of sterile saline. An old colony is perhaps preferable where there is practically nothing left but spores. After vigorous shaking a sample of this suspension is transferred to a haemocytometer stage, or preferably to a counting chamber such as has been devised by Professor Ernest Glynn for the numerical estimation of bacteria in vaccines. Having ascertained the number of spores or bacilli per c.c. in the suspension, it is an easy matter to make from this a dilution which will contain approximately one spore or one bacillus per unit of saline. When the desired dilution/

dilution has been obtained, it is an easy matter to sow a series of meat or Hirnbrei tubes each with the unit volume of saline containing a single organism or spore. These are then incubated anaerobically, and thereafter examined as to their purity. If a sufficiently high dilution has been made there is every probability that a pure strain or strains will be secured.

The method is simple, does not take up much time, reduces the possibility of extraneous contamination to a minimum, and, short of raising a culture from a guaranteed single bacillus, as the ideal method would be, offers a very reasonable prospect of success.

Two attempts were made before I was successful in isolating the organism I wanted. On the first occasion I only succeeded in isolating the slender round spored bacillus, but at the second attempt I was able to get what I now regard as probably a pure culture of the bacillus. Its morphological and cultural reactions I shall describe further on in the course of this thesis.

The oval-sporing organism thus recovered when introduced into a sterile tube containing minced bullock's heart and incubated anaerobically at 37° Cent. in two or three days emitted the characteristic wound odour, while the meat began to blacken and diminish in volume, indicating active proteolysis. This, as I have/

have said, at once suggested the possible rôle played by this bacillus in the case of wound infections, viz. that under suitable conditions for active growth and proliferation it did in the dead tissues of the wound what it appeared to do in vitro, viz. it digested them.

Looking at the organism from this point of view, the conclusion suggested itself that it was in reality the active and probably the main agent concerned in the cleansing of wounds which did well under the salt bag technique, and where it was absent, the wound remained refractory to this method of treatment, and simply resembled one which was being treated with one of the usual antiseptics rather than with salt packs.

As the organism, however, belonged apparently to the same group as the *B. Oedematis Maligni* and the *B. Tetani*, it was still regarded with suspicion in view of the universal terror of spore-bearers with which the wounds received during the present war has inspired us, and in view of the natural desire to rid the wound of such with all possible haste. In my opinion, it is owing to the extreme virulence of some of the members of the anaerobic group that all have been condemned indiscriminately.

This, however, is as it may be. It was necessary to find out if this particular bacillus which I had isolated/

isolated possessed pathogenic properties or not.

To this end I inoculated various animals with varying doses of culture made on different media, and grown anaerobically for varying lengths of time. In no instance was any ill effect noted. Further, as some of the cultures used were likely to contain the toxins, if any, produced by the organism, animal inoculation was calculated to show evidence of such. In no case was there any proof of the existence of such a toxin.

Further, as it was apparently present, judging from the characteristic odour, in all those cases which pursued a normal course, I examined the bacterial flora from a certain number in the manner already referred to, and was almost invariably able to isolate a similar organism.

Taking into account, therefore, the lack of any evidence of pathogenic effect on animals, together with the fact that it was probably present in all those wounds which gave out the characteristic odour and which were also doing well under the treatment, and that, further, its presence had been definitely proved by its isolation from many of those cases, I made the tentative suggestion to my surgical colleague that
it/

it would be legitimate actually to introduce this anaerobe in living culture into some wound which had previously been subjected to salt bag treatment, but which had failed to smell or to improve, and from which I had been unable to recover a similar bacillus.

Such a case in due course presented itself. It had been treated in the usual way with salt packs, but it had failed after some days, either to smell or to improve. Cultures which I made from this wound failed, moreover, to reveal the presence of the particular anaerobe under discussion. My Colleague agreed that an attempt should be made to infect this wound, and on removal of the salt packs which had been inefficacious, I was present in the operating theatre and immediately sowed the wound with the contents of a meat broth tube containing a culture of the living organism. The wound was thereupon packed by my colleague with salt bags in the usual way. The contrast between the effects produced by the first and second salt packings respectively where the only factor of difference was the presence of the spore-bearer on the second occasion, was most remarkable.

In three days' time, the patient's temperature had come down, the wound was emitting the foul and characteristic/
istic/

characteristic odour associated with the presence of this particular anaerobe, and the patient was comfortable. Within a few days after removal of the second salt packs, the wound was absolutely clean, devoid of all sloughs, a brilliant scarlet colour like fresh beef, and was covered with healthy granulations. This case was its own control. It had previously been packed in the usual way with salt bags, it did not develop the characteristic smell, the patient did not improve, and this particular type of spore-bearer was absent in culture. It was then repacked with salt bags as before with one added factor, viz. the introduction of the spore-bearer in question and this time with complete and rapid success.

In the light of these facts I concluded that the organism which I had introduced was in all probability the vital factor in determining success on the second occasion of packing. Since this first case was so treated, others have been encountered in which the salt bag did not alter the patient's condition for the better, and which did not smell. These have been sown in the same way with living cultures of this particular spore-bearer, after first making cultures from the wound to determine definitely its absence from that wound before sowing. Such wounds have then been repacked with fresh salt bags in the usual way, have invariably/

invariably done well and have afforded olfactory proof of the active presence of the organism. The question next arose as to the rôle played by the salt in the successful treatment of these wounds. Was the salt really necessary, and did the success met with in this form of treatment depend not merely on the presence of the bacillus, but also on the action of the salt as described by its chief exponent, Wright?

Having regard to the anaerobic nature of the organism, I felt that possibly the tight packing with the salt bags, together with the subsequent outflow of fluid and the collection of pus that ultimately collected in the packing acted rather by reason of its rendering the wound more or less anaerobic than by any inherent virtue possessed by the salt either as a germicide or as a lymphagogenic agent. To determine this point I suggested to my colleague that in place of salt it might be worth while to substitute some absolutely inert substance such as sterilised sphagnum moss. He agreed to this and had bags made similar to the salt bags in use, but in place of salt, sphagnum moss was used.

Certain cases were chosen, sown by me with a living culture of the organism, after which my colleague introduced into the wound bags containing only sphagnum moss, in such a way as to lie in contact with every/

every part of the wound completely filling up the cavity. The technique was the same as that hitherto employed in salt bag treatment, with the difference that an inert substance was used instead of salt.

It was recognised that sphagnum moss would probably be an ideal packing for such wounds, since, with the accumulation of pus and fluid, the moss would swell out and so mould itself more closely to the shape of the wound, while at the same time all interstices would become filled with fluid. By this means I hoped to be able to render the wound more or less anaerobic and so afford the most favourable conditions for the growth of the anaerobe, while the salt factor would be eliminated.

As I anticipated, the cases so treated followed exactly the same course as those sown with the organism but where the usual salt bag had been employed for packing.

Hence I came to the conclusion that salt in this form is neither essential in the treatment of these wounds, nor is it necessary for the cultivation of the specific anaerobe, and with the recognition of this fact must disappear many of the intricate and elaborate theories built up by Wright to explain its action. Where salt packs are employed they seem to act chiefly by/

by rendering the wound anaerobic, so providing favourable conditions for the growth of the particular organism under discussion where such organism happens to be present. In the latter case the organism soon gets to work, and the wound rapidly begins to improve and goes on to satisfactory healing. Where this particular anaerobe is absent, the salt pack, in spite of the salt and all its lymphagogic action, is useless as an agent for cleansing the wound or for improving the patient's condition.

One factor and one factor alone seems necessary for success, viz. the presence of the organism or of its spores, together with an environment conducive to its growth and multiplication. Fortunately, this particular bacillus or more likely its spore form is present in a large proportion of the cases, being implanted along with pathogenic organisms probably at the time when the trauma is inflicted. As its presence, however, cannot invariably be reckoned upon, it ought to be artificially sown as a matter of routine, and such a procedure is easy of application. Since it is an anaerobe and a spore-bearer, supplies of it can conveniently be distributed in small ampoules or glass capsules, one or more of which could be broken over the wound, thus liberating the living culture. Such ampoules/

ampoules I have put up for distribution. It is true that no acute cases of gas gangrene have been available here on which to study the effect of this method of treatment, although the wounds so treated have been both large, filthy and sloughing. I cannot therefore say from personal knowledge how this particular anaerobe would act in such cases of gas forming bacillary infection.

I can, however, point to the published experience of certain observers who have used the salt pack method of treatment with marked success in such cases. Roberts & Statham, in their paper on the Salt Pack Treatment of Wounds, ⁹⁾ publish brief descriptions of seventeen cases of wound infection treated with salt bags. Six of these cases were examples of gas-infection. All improved rapidly under this treatment, with the exception of one which at first improved and then flared up so that recourse was had to amputation. Perhaps I may be allowed to recapitulate briefly the details of one of their successful cases. The patient was admitted on May 25th, 1916, with wounds of the right leg and left thigh, both infected with gas-forming bacilli. There was a small superficial entry wound in the left calf running deeply in with a tube in it. In front of the outer side of the left thigh was a wound/

wound the size of a shilling; great oedema of muscles, while stinking pus and gas oozed out. Operation was performed the same day. This apparently consisted in laying the wound in the calf freely open - cutting away much infected muscle, thus leaving a cavity 6 inches long with exposed bone and in packing this cavity with salt and gauze. The thigh wound was treated in the same way. The patient was collapsed, jaundiced and vomiting and was placed on the "dangerous" list. On the day following the operation the patient's temperature was 102° , but the pulse was only 86. He was still troubled with vomiting. B. Perfringens was freely grown from the pus. On the 3rd day great improvement was reported, the patient was eating well and the temperature only 99.2° . On the 7th day after operation the wound was repacked for the first time, when it was reported to be much cleaner. It was again dressed 9 days after the second packing, i.e. 16 days from the date of introduction of the first salt packs, when it was now found to be quite clean and granulating. The wound was then strapped and four days later the patient was sent to England practically healed. Such evidence leads me to believe and certainly to hope that in acute cases of gas infection, the presence of the specific spore-bearer which I describe further on in this thesis, together with salt or sphagnum moss packing, /

packing, is likely to be attended with the same good results as have been undoubtedly obtained here in the case of septic wounds which showed no clinical evidence of gas infection.

I come therefore to a point where, from the reasons given in the preceding pages based on bacteriological findings and on actual clinical experiment, I feel justified in venturing to advocate a method of treatment for septic wounds which so far as I know is an entirely new one, and one whose possibilities have never hitherto been exploited. To do so may at first sight appear not only daring, but heretical, but I can only point to my line of argument and to the clinical results achieved, and ask that a fair trial be accorded to the method, following the directions laid down in this thesis. It is a method, further, which involves the simplest of technique, the simplest and cheapest of dressings, since salt or sphagnum moss are easy to procure, and is one which causes least disturbance to the patient. It is a method which does away with the necessity for frequent dressing, so obviating the mental and physical suffering entailed thereby.

To revert again to that which has impressed me most in connection with wounds inflicted during the present war, viz., the possibilities for evil that exist/

exist in the dead or devitalised tissues where pathogenic organisms are present, it is necessary to emphasise once again that the method of treatment which I now advocate seems to depend largely on a recognition of the sinister and important role which these dead tissues may play.

Since modern methods have been used in the treatment of wound infections, they have almost invariably been directed towards the extermination of the infective agent. The main difference, perhaps the only one present to the minds of the average surgeon, between an aseptic wound and a septic one, was the presence in the latter of bacteria. To destroy these pathogenic organisms was his first consideration, and he tried to effect this by a mass attack on the bacteria themselves, with antiseptic chemicals which had previously been proved capable of killing bacteria in vitro. As this method, however, has been found in actual practice to be attended only with partial success, it was argued that a better way to attack the pathogenic flora was to induce the body cells to take up their own defence. Much was expected from this method, but it in turn was found to yield ambiguous and often disappointing results. Recognising that neither one nor the other of these methods could be implicitly relied upon to succeed in every case, the surgeon has become impatient of them all, /

all, and has turned as a last resort to the scalpel, with the avowed intention of removing en masse the infecting bacteria, both superficial and deep, and obviously this could only be done by removal of the bulk of the devitalised tissue. The results, where this method has been practicable, have been good, although the procedure has involved further mutilation of the patient: Now, as I have pointed out, the reason for the partial failure of the first two methods, and for the comparative success of the third probably consists in the fact that whereas the advocates of the antiseptic and of the physiological school respectively concentrate their attentions on the pathogenic bacteria present, the surgical school in their attempts to combat the infection, actually remove not only the greater part of the bacteria, but also the devitalised tissue. The removal of this is the chief point of difference between these various methods.

In a surgical wound which has been kept aseptic, the time required for complete repair will depend to a certain extent on the amount of dead tissue present. In a septic wound it will furnish a base from which pathogenic organisms may harry the body of the patient. The dead tissue will furnish more or less abundant pabulum according to its amount, which will provide for/

for the rapid proliferation of the bacteria present. Apart from any exo-toxins which these bacteria may produce or any ⁿendotoxins which may be liberated on the death and breaking up of the bacterial bodies, the vital processes involved in turning this pabulum to account may liberate chemical substances which have an inimical effect on the human body. As illustration of the latter suggestion I might quote the investigation carried out by Barger and Dale ^(10.) to determine whether the bacillus of malignant oedema actually did produce a toxin and if so whether it was a specific one. As a result of their enquiry, they came to the conclusion that while the filtrate from cultures of this bacillus possessed properties toxic for animals, yet the toxin was in point of fact not a specific one. The toxic properties of the filtrate were really due to the fact that in the course of organismal growth and proliferation, the proteins contained in the culture were rapidly broken down to amino-acids and from these the amino-groupings were split off, so yielding ammonium salts of the fatty acids which were apparently responsible for the acute symptoms observed in the experimental animals.

Bacteria, I would suggest, therefore, require sufficient pabulum for their successful growth and proliferation./

proliferation. This, as a rule, is an easy matter in vitro and, given a proper adjustment and supply of the pabulum, will go on indefinitely because unhampered. In the human body, on the other hand, it is quite another matter, since the body cells are endowed with a complex mechanism of defence having for its object amongst other things, the destruction of pathogenic organisms which threaten the well-being of that body. The unfettered growth and proliferation of bacteria are thus held in check by this defensive system, and unless the latter be naturally or at the time of attempted bacterial invasion, imperfect, or unless the mass attack by the bacteria be overwhelming, the body is quite able to conduct its own defence and to destroy the invading organism. It is quite another matter, however, when a part of the body has been wounded. This, as I have indicated, means that a certain part of the wounded tissues have been killed. The bacteria introduced at the time of the injury or later have, at their disposal, tissues which no longer possess the full powers of defence which were theirs in common with the rest of the body before the infliction of the trauma. Such devitalised tissue is much more favourable territory for bacterial colonisation than are the uninjured tissues of the body, and the bacteria very properly make use of it. It is, however, not such/

such a favourable breeding ground as, for example, the medium contained in our culture tubes, since the proximity of the living tissues to the dead allows a certain amount of scope for the body's defensive mechanism to come into play in the shape of phagocytes and bactericidal substances. The body, however, is at some disadvantage, for whereas a single or occasional attempt to invade it may be easily repulsed by the body's defensive mechanism, yet when a base has been established in the shape of devitalised tissue, it is quite another matter to deal with the more or less persistent invasions that may be made into the body from that base. It seems to me that the danger lies not so much in one mass attack as in repeated small attacks made by the bacteria or their toxic products, continued over a period of time. It may be compared to the "wearing-down" tactics in warfare with which we are unfortunately acquainted at the present time. The danger would be entirely eliminated or, at least largely minimised if one could destroy the base from which these hostile activities proceed. The danger to the patient will depend largely on the length of time we allow that base to exist, presuming that it is already infected with virulent organisms. It will also depend on the volume of dead tissue in the wound, especially/

especially where organisms are present which, while they do not actively produce a specific toxin, nevertheless give rise to toxic substances in the course of their biological activities. Presumably the dose of such or the length of time over which a supply can be thrown into the body will depend on the mass of dead tissue left after the injury not to talk of that which may die after the initial trauma as a result of mechanical factors or of bacterial activities. Hence the importance which I attach to the presence of dead or devitalised tissues in a wound. It will be seen from this conception of wound infection why antiseptics should so often fail, and why they have come to be regarded with some degree of disfavour. Whatever their lethal power when exposed to bacteria in suspension in test-tubes, that lethal power is greatly diminished when it comes to be a question of exterminating the organisms present in a wound where there must be dead tissue, and where the environment is wholly different. Many of the superficial bacteria are doubtless destroyed, and it may even be that growth on the surface is inhibited for a time, but unless the antiseptic is to saturate the devitalised tissue, the bacteria in the deeper recesses will go unscathed, and will go on bombarding the patient with toxins or toxic products./

products. The time element in antiseptic treatment is also not without importance from two other points of view. At the present juncture it is important that the period of convalescence be reduced to the minimum. With the antiseptics hitherto used it is doubtful, to say the least of it, if this can be done. We see over and over again cases treated with antiseptics that have gone on suppurating for weeks. What is more important from the patient's point of view is that the longer the dead tissues are allowed to remain or are added to by strong antiseptic applications, the longer is the body exposed to the continued action of the bacteria and their toxic products, which in time may exhaust the defensive mechanism, and finally overwhelm the patient. Further, as I shall show later, it is possible for an organism to accustom itself to resist increasing concentrations of a given antiseptic. Hence, if the same antiseptic is persistently used, it may actually begin to lose what power it originally possessed of damaging even the organisms on the surface of the wound. Further, many antiseptics will themselves cause death of the tissues to which they are applied. The action of such, therefore, so far as the tissues are concerned is to perpetuate the devitalised conditions, so extending the area suitable for/

for bacterial activity. This does not mean for a moment that antiseptics have no place. They seem to be comparatively efficient in many types of wound, but their efficiency in these cases does not depend so much, perhaps, on the kind of antiseptic employed, as on various other factors, such, for example, as the type or combination of bacteria present, the amount of dead tissue, etc. In the bulk of the war wounds met with at the present time, however, there is an increasing concensus of opinion that treatment by the antiseptics hitherto in common use have not yielded the results we were led to expect. In talking of antiseptics in this thesis, I wish it clearly to be understood that I do not include Browning's acriflavine or the hypochlorous acid solutions recommended in the first instance by Lorrain Smith and his co-workers, and later by Dakin, - acriflavine, because I have not so far had any opportunity of investigating its behaviour in wound infections and hypochlorous acid solutions for a reason to which I shall refer again.

What I have said in regard to antiseptics is true to a certain extent in the case of the physiological method. Here the attempt is made to attract the body cells, lymph, etc. to the damaged part so that they may deal with the infection. The attack, be it/

features of gunshot wounds have been extensively investigated by Bashford¹¹⁾ in a deeply interesting article which demands careful study. In the course of this paper he states that the bacterial invasion ceases or is stayed where the circulation is maintained unimpaired, but that under the influence of bacterial products, this zone of degeneration is seriously threatened. In view of these and other facts, and with evidence before him that the products of the bacilli continue to inflict damage on the vessels, muscle fibres and endomysium ahead of their massive advance, he seems driven to advocate surgical interference as the only sure means by which the products of organismal activity will be prevented from continuing their ravages by damaging and finally killing fresh tissue. This is apparently another way of saying that the wound with all its dead and devitalised tissues should be excised at the earliest possible moment. Bashford's conclusions, however, are mainly based, as I have said, on histological evidence, and he accepts as potential agents of destruction, all anaerobic bacilli without any discrimination which he sees in the tissues.

Our whole knowledge of anaerobes, however, appears in the light of recent bacteriological work, to be more or less imperfect and chaotic, and much still remains/

remains to be done before we can appreciate their true significance. So many of them produce such potent poisons as a result of their activities on dead tissue, that all are regarded with just suspicion, and for that reason are indiscriminately condemned. The particular organism whose cultural characters, etc. I shall describe further on appears, however, to be an exception. So far as I am able to judge, this organism is non-pathogenic, does not produce toxins inimical to the human body, and does not attack living tissues. At the same time, however, it appears to be able rapidly to break down dead tissues without, in doing so, producing toxins capable of acting detrimentally on the living structures. Some proof of this is afforded, amongst other things, by the appearance of the shreds of slough remaining after removal of the salt packs from a wound which has been successfully treated. They look thin, transparent, and soon wash away under slight irrigation. This organism appears to be able to dissect out not only the macroscopically but also the microscopically dead tissue in a way that no surgeon's knife ever can, and that without at the same time inflicting any fresh trauma. The devitalised tissues are practically all removed in the course of a few days, and with their disappearance, the breeding ground/

ground of the pathogenic germs is destroyed. The living tissues, relieved of the strain of ever having to combat a continual bombardment from bacteria and their toxins, while endeavouring at one and the same time to cast off the dead slough, are now able to throw all their energies into the work of repair, as is evidenced by the rapid formation of healthy, strong granulations which soon form an efficient barrier against further organismal attacks.

While, however, I have laid considerable stress on the proteolytic activity of this organism in relation to the dead tissues, since it is highly probable that this is the fundamental principle involved, I am aware that other bio-chemical factors may be involved especially in view of the almost immediate and rapid improvement in the patient's general condition. This improvement in a successful case becomes pronounced about the third day, and from what we know of its biological characters seems to be due to the activity of this particular organism. It cannot wholly be due to the removal of the dead tissues, for even when the salt packs are removed in 7 or 8 days' time, there may still be some shreds of slough remaining. What these other factors are, can only be surmised at present, but I shall refer to them later when I give details of/

of the organism.

The removal of the dead tissue appears to be the first and essential step towards ridding the wound of infection and, of all the antiseptics which have been advocated and tried, none appear to do this with the exception of the hypochlorous acid preparations. These, out of all the antiseptics in use in Reading, seem, so far as I can learn, to have given the best results. This also appears to be in accord with the published statements of those who have used this method extensively. Is this due to its antiseptic action pure and simple, or is there some other explanation? Those who have been in the habit of employing Eusol in Reading tell me that it causes the separation of sloughs more rapidly than any other antiseptic they have used. Dakin, in one of his papers, "Biochemistry and War Problems", ^(12.) states that "the solvent action of "hypochlorites on necrotic tissue is a great advantage "when contrasted with the coagulating effect of many "antiseptics on blood serum and wound exudates. "The former action of hypochlorites permits the wound "surface to remain moist and so removes obstacles to "the outward flow of lymph which is so readily checked "by antiseptics which are protein precipitants."

Again, /

(13.)

Again, in "Studies in Antiseptics" he says, talking of chloramine, that "the results were clinically similar to those observed in the early treatment of infected wounds with sodium hypochlorite, with the exception that sloughs are dissolved somewhat more readily by the hypochlorite than by the chloramine." It is not quite clear from those statements whether Dakin regards the beneficial action of hypochlorites as due to their antiseptic properties or to their action in dissolving away sloughs, but the following is unequivocal.

In the course of a discussion following a paper on the Secondary Closure of War Wounds, read at a meeting held at the Paris Academy of Medicine, M. Dastre and others expressed the opinion "that the beneficial effect of hypochlorite was due to its ability to clear away damaged and necrotic tissue, and to destroy toxins rather than to its antiseptic action." (14.)

To quote the experience of one other, Fleet-Surgeon Dalton, R.N. (15.) gives as one of the advantages of the use of sodium hypochlorite solution, "the rapidity with which sloughs separate and clean granulation tissue is formed in a wound under its influence."

A review of these statements seems to indicate that hypochlorous acid solutions do not act merely by/

by virtue of their antiseptic properties, but that combined with these is another - the ability rapidly to remove dead tissue. It may well be that this latter is the most important effect of the application of hypochlorous acid solutions, and that this is the real explanation of the good results obtained.

Again, in the columns of the Lancet,^{16.)} reference is made to a paper appearing in a German Journal, in which a certain German surgeon describes his experiences with artificial gastric juice in the treatment of gangrenous wounds. Unfortunately no details are given and no mention is made of the particular Journal in which this appeared.

I have thought it well to take notice of these references, since they show that the dead tissues are claiming more and more attention at the hands of surgeons, and in so far as a given method of treatment succeeds in destroying or removing quickly the necrotic materials, to that extent it can be claimed to be of greater value than other methods which do not directly act upon the dead matter. On the assumption that it is the latter whose rapid removal constitutes the first step towards safeguarding the patient's life and towards enabling his own defensive mechanism to take up the work of protection and repair under the best possible/

possible conditions, we must acknowledge that neither the ordinary antiseptics nor the newer physiological methods achieve this purpose.

Of those which bring about separation of the dead material, whether of set purpose or as part of their general action, there are the two methods to which I have referred. One is the surgical and the other may be termed the chemical, viz. by means of hypochlorous acid solutions. The objections to and limitations of the former have already been referred to. The drawbacks which attend the use of the latter seem to me to be twofold. In the first place, for the chemical to be efficient, there must be frequent redressing of the wound - a proceeding which is bad for the patient, since it breaks the cardinal rule that a part which is injured demands rest - apart altogether from any secondary effect it may have on the mental condition of the patient. In the second place it seems to provoke unnecessary bleeding, which, although in many cases unimportant, is liable to become a matter of some concern.

In place of either of those methods, with what I consider their limitations and disadvantages, and without in any way wishing to sit in judgment upon them, I desire in this thesis strongly to recommend an/

an entirely new method of wound treatment. This method, the principles of which have been described in these pages, I should like to call the biological method. I come forward as the advocate of this method which has now been extensively tried in this hospital, not so much because I think that the methods by excision or by chemical agency are bad, but because I think the new method infinitely better. Without inflicting fresh trauma on the wounded tissues, without causing further mutilation of the parts, without in any way inducing further haemorrhage, it is the means of removing rapidly all dead tissues in a way that excision never can, and in addition causes in some way an almost immediate and miraculous improvement in the patient's general condition. Since it is a "living" method, its action goes on progressively and automatically as long as there is a supply of dead material, and, once introduced into the wound in the appropriate way, there is no further need to disturb the dressings.

This stands out in marked contrast with the chemical method where, the range of potential action being limited and soon exhausted, recourse must be had to frequent re-dressing. The difference consists in the fact that in the biological method, the proteolytic action is being constantly exerted, being an attribute of/
of/

of the living organism implanted, whereas in the chemical, this action, where it does exist, is limited, soon exhausted and without the power of reproducing itself indefinitely. That method which will be most conservative of human life and limb, which will curtail the convalescence of the wounded man, which is easy of application, and at the same time requires least attention while withal it is free from danger, will surely be acclaimed the best method of treatment. That method, I venture to submit is the one which I advocate in these pages.

In the treatment of war wounds by this biological method, however, there are certain practical points to which it is necessary to draw attention, and they are points of some importance.

In the first place, I would emphasise as strongly as I can, the fundamental necessity of laying open a wound in the most thorough manner possible. It is a procedure that is often neglected, but it ought to be laid down as one of the first cardinal principles in the treatment of war wounds. Whether obviously dead tissue is cut away at the same time or not, is, with the method of treatment which I now advocate, not absolutely necessary. The all-important point to keep in mind is that every pocket should be thoroughly laid/

laid open if possible, and that the subsequent packing should lie in contact with every part of the wound.

As the wounds are so often of an irregular and burrowing character, this will call for the ingenuity of the surgeon in each individual case. All foreign bodies must obviously be removed, and at the same time, without resorting to the extreme measure of excising the wound "in toto", all grossly damaged and obviously dead tissue may be detached.

The interior of the wound is now irrigated with sterile water or saline to wash away obvious pus or blood, and the living culture of the organism to be described later, is now sown in the wound, for which purpose I have hitherto used a pipette.

Immediately thereafter the packs, whether of salt or of sphagnum moss, are introduced into the wound so as to lie in contact with every part of it. These packs, just before their introduction, are moistened with sterile water or with normal saline solution. Over all, several layers of plain white gauze are laid after moistening them in the same way. The wound is thus completely and firmly packed, and, after covering with a thick layer of cotton wool, the whole is firmly bandaged. Where possible, it is an additional help if an outside splint can be applied to aid in steadying the/
the/

the part. The packing itself, however, acts as a very efficient splint as we shall see later.

Where salt has been used, the patient often complains for a few hours of slight pain and smarting, and whatever form of pack is used, the temperature will probably rise even higher the next day than it was before interference with the wound.

In the course of the first twenty-four hours, where salt has been used, there is a very copious discharge of fluid, due doubtless to the strong concentration of salt in the wound. Thereafter the flow ceases again, presumably because much of the salt has been carried away by the initial outflow, and what remains is probably in low concentration. A certain amount of pus oozes away, and towards the end of the second day, or at latest the third day, the wound begins to smell in a characteristic manner in those cases where the organism or its spores happen to be present. This corresponds more or less closely with what occurs "in vitro". A culture sown in meat broth from the sporing form does not begin to smell much till the end of the second or till the third day, indicative probably of the length of time needed for the spores to germinate sufficiently for the proliferating organisms to attack the dead tissues. At the same/

same time, as has been noted by nearly all who have written about the salt bag method of treatment, the temperature, after an initial rise, begins to come down also towards the end of the second or on the third day, after which it generally remains down. The time taken for the temperature to drop seems to coincide with that which elapses before the characteristic odour becomes marked. In some cases, however, the temperature does not drop, although the organism is active, judging from the smell. In such cases, experience has taught that it is not the packed wound that is at fault, and, lest the method advocated should be condemned on the strength of one or two such cases, it is necessary to make the following statement.

If in an unsown case where salt or sphagnum moss bags have been employed, the temperature, by the third or at latest the fourth day, shows no signs of subsiding, and where the characteristic and offensive smell is absent, the wound is not doing well and is not going to do well under that treatment. It is a signal for sowing the wound with a living culture of the organism, after which it should be repacked.

Where, on the other hand, the characteristic smell is obviously present, indicating that the organism is doing its work, and yet there is no fall in the temperature/

temperature by the third day, look out for some other focus of infection or some other wound which, perhaps because of its apparent insignificance, has been treated in some other way.

From the third day onwards in a successful case, the patient steadily improves. There is no daily dressing to worry him, except perhaps the laying on of a fresh pad of cotton wool. He has no pain and is able to eat and sleep well. There is only one objectionable feature, and that is the foul odour, which however is a sign that all is proceeding satisfactorily. While the temperature is said by some to be no criterion of the patient's general condition so long as the pulse remains good and the patient feels well, I venture to disagree for the reasons stated above. Due attention must be paid to the temperature as well as to other points.

Next comes the question of how long the pack is to be left in situ before attempting to remove it. In order to get the best result, further observation on this point is necessary, but probably it ought not to be less than seven days, although this may require to be regulated according to the type of wound. No general anaesthetic is necessary when the time comes for removal of the packing. The superficial layers of/

of gauze will be found more or less firmly adherent to the skin and set hard like a board. Exactly what is the stiffening agent need not be discussed here, although it is probably of an albuminous character. Suffice it to say that it has been observed by all who have written about the salt bag method.

After gently detaching the board-like upper dressing from the skin, the rest of the packing, what were the salt bags originally, comes out easily en masse, bathed as a rule in bright yellow pus, from which the proteolytic organism can be recovered. A few more or less delicate sloughs may still remain adherent to the wound surface, but, on irrigation with warm saline or Eusol, they either become detached at once or do so in the course of a few days. As the irrigating fluid pours over the surface, these wave about like little fragments of transparent sea-weed. The wound is then lightly dressed with plain gauze wrung out of normal sterile saline, and thereafter once a day. In the course of one, two or three days, the exposed muscle will become a brilliant red, devoid of sloughs, and covered by firm healthy granulations. All oedema and redness have disappeared from the surrounding tissues.

Such a wound heals rapidly, can be brought together/

together with strapping or skin-grafted, and, when finally healed, presents a beautiful, firm and more or less linear scar.

One point deserves special mention, and that is, that in no case where this method of treatment has been employed here, has secondary haemorrhage ever occurred. So noteworthy is this feature that it has been remarked upon by others who have followed the use of this method. Major A. J. Hull, R.A.M.C.¹⁷⁾ even goes the length of saying that in his hands the salt bag method of treatment has been one of the most generally applicable of procedures for the treatment of secondary haemorrhage. This seems to be in direct opposition to the published statement^{18.)} of Sir Almroth Wright, who has said with reference to secondary haemorrhage, that the aim and object of treatment must be to prevent any digestive action in the neighbourhood of the endangered artery!

One further point deserving of notice has been observed by my colleague, Major Joyce, viz. that sequestra appear to loosen and come away more rapidly in cases treated with salt bags than in cases not so treated. When they do come away they look white, clean and devoid of all fragments of soft tissue. Further, he has noted that where from anatomical reasons/

reasons it is necessary to carry out manipulative procedure for the removal of sequestra, no post-operative flare-up occurs such as one generally encounters after a similar procedure in cases that have been treated in some other way. The reason for this may be that, in the former type of case, all dead soft tissue has been completely proteolysed so that the breeding ground in and round the bone for lurking pathogenic bacteria has been destroyed. In the second type of case, on the other hand, both are present, and slight manipulation even may light up fresh trouble.

Having thus, at some length discussed the theme of this paper, I shall now deal with a few clinical cases, after which I propose to give a description of the specific anaerobe with its cultural characters, together with notes on any experimental work carried out in connection with it.

CLINICAL SECTION.

All the cases which have undergone salt-bag treatment in Reading have been under the care of one or other of my colleagues, who have been kind enough to place at my disposal for reference the case sheets of their patients. As, however, I have been able to follow the clinical progress of most of those cases before and after treatment, and to make personal notes and observations thereon, I have not been under the necessity of relying solely on the clinical observations of others.

To give a detailed account of all the cases treated by the salt-pack method would make this thesis unnecessarily long without at the same time rendering it any the more convincing. I have, however, recorded in the following pages a series of cases by way of illustrating the points dealt with in other parts of the thesis. The brief summaries which follow are based partly on the clinical records and partly on my own notes and observations, and in a commentary appended to each I have discussed the significance of the events in each individual case.

While the cases are not all of one type they all agree in being the result of gun shot injuries. From some points of view it might be preferable if in each/

each case exactly the same treatment had been adopted. For example if every wound had in the first instance been treated with salt bags without an ensuing clinical improvement and without furnishing bacteriological evidence of the active presence in the wound of the Reading bacillus, but had made a speedy and uninterrupted recovery after that organism had been implanted, without further interference beyond the insertion of fresh salt bags, such evidence might well be considered flawless. There exist, however, very obvious difficulties in the way of submitting every patient to the above routine treatment, however desirable it might be from an experimental point of view,

First of all the cases are under the charge of the clinician, who is responsible for their welfare and who must carry out what measures he deems necessary as the need for them arises. A patient may be so seriously ill or may have undergone so many previous operations that the surgeon has, in the one case, no time to treat the wound experimentally, and in the other, in the interests of the patient, no inclination. Such must always be the case where new methods are still on trial.

In the second place the treatment of a wound is not on all fours with a test tube experiment. In the latter one can control at will the various factors/

factors. In the former this is only partially possible. Apart from the instances just mentioned where, for clinical reasons, all the steps of the experiment cannot be carried out in the order suggested, there are others. For instance, when the surgeon exposes a wound for the reception of the Reading bacillus an abscess may have formed during the time that the first set of salt bags were still on trial. This must obviously be evacuated. Again he may discover loose fragments of bone which he will almost certainly remove. Further, in many cases the Reading bacillus is already actually present in its spore form and only awaits suitable conditions for its development and growth such as would be provided by the introduction of the first or preliminary set of salt bags.

Nevertheless, notwithstanding these difficulties, I have been able to include a few cases which fulfil the rigid conditions above enunciated, and which consequently may be regarded in the nature of critical experiments.

It is moreover not altogether unfortunate that all the cases are not exactly similar. Some of them serve as controls, while all of them by their very differences throw into stronger relief the actual processes which are at work in cases treated with the Reading/

Reading bacillus and go far to corroborate the value of the method advocated. I have purposely arranged the cases so that they fall into definite groups, each group illustrating one or other of the points raised.

In the wounds which get well under this new form of treatment, there are one or two factors, in the cases cited, which may be regarded by some critics as responsible for the improvement. The latter it may be urged is due in some instances to operative measures carried out on the occasion of packing the wounds. Some abscess which had formed, has been opened, some foreign body extracted, or some fragment of loose bone removed. It is impossible to deny that such interference may not only be necessary but also beneficial. In estimating, however the degree of importance to be attached to such measures, one must bear in mind that operative procedure of such a nature is frequently undertaken without any corresponding benefit. Not once only but many times in the course of a wound's history may operative measures of a minor character be required. The very fact that they are so often necessary affords proof that the method of treatment adopted for the cleansing of/

of the wound, is to that extent lacking in efficacy. Reference to some of the cases here recorded will afford instances of what I mean. It is undeniably most necessary that such measures should be carried out but having done so, rapid improvement does not invariably ensue.

Why is this? The reason, in my opinion must be sought in the fact that there are still left behind in the wound dead or devitalised soft tissues together with the pathogenic organisms which find therein a means of subsistence. As a consequence of the vital activity of these organisms on the dead tissue, toxic substances are produced which find an entry into the patient's system. The result is a lowering of his powers of resistance so that in the end either the patient succumbs or, if recovery does take place, it is a long and tedious one, punctuated perhaps by minor operations from time to time. The very fact that abscesses require to be opened or fragments of bone removed more than once in the course of a wound's history, indicates that such measures are not by any means the real factor upon which ultimate healing depends. The very fact that such minor operative procedure has to be repeated time/

time and again in the same case, seems to prove that the necessity for them is really the result of something else that has never been perfectly eradicated.

The abscesses which form on the fragments of bone which necrose are merely the resultant of organismal forces acting on tissue already dead. To open the abscess, or extract the sequestra is simply to remove part of the effect without the cause. So long as the latter is allowed to remain there exists a strong probability that fresh abscesses or further sequestra will form.

That this, as I have stated elsewhere, has come to be more fully realised is proved by the fact that many surgeons now favour early excision of the wound. It is interesting to note that in a recent article in the British Journal of Surgery, the authors advocate independently, as I learn from a personal communication, a somewhat similar view when they state that probably "along this line - namely, rapid destruction of dead tissues, - lies the way to further advance in the treatment of wound infections."

Complete removal, then, of all dead tissue, not merely some part of it, together with the infecting organisms ought to be the goal at which to aim.

To excision by the knife there are many obvious limitations/

limitations to some of which I have previously made reference. As a more efficient substitute I feel justified, both on clinical and experimental grounds, in advocating what I have elsewhere named the biological method, depending solely on the vital activities of the Reading bacillus.

The point I have tried to labour here more particularly is this, namely that where it was found necessary, in some of the cases about to be mentioned, to perform some minor operation at the time of sowing with the specific anaerobe, the subsequent improvement in the condition of the patient together with the healing of the wound ought not to be attributed to such operative measures.

This contention is supported by the fact that many cases which had failed to improve under other methods of treatment, when sown with living cultures of the organism without any preliminary operative procedure beyond the possible enlarging of the existing wound in order to admit of adequate packing, cleared up exactly in the same way as those on which a preliminary minor operation had been performed. These cases are recorded in a separate group. Moreover if further proof were required, it might be found in the fact that the subsequent history of wounds/

wounds which have been most thoroughly explored and submitted to operative interference as well as to subsequent antiseptic treatment, is entirely different from that which characterises wounds successfully treated by the salt bag method. That this is so does not depend wholly on my own observations, but receives corroborative support in the papers of quite independent workers, to whom reference has been made elsewhere in this thesis.

The clinical course of a successful salt bag case is in a category by itself - it is *sui generis*.

I have already referred and shall have occasion to do so again, to the rapid amelioration which ensues in the patient's general condition as evidenced by his subjective symptoms, his pulse and his temperature. This constitutional improvement may be reckoned almost in terms of hours, while within a comparatively few days the wound itself has become a healthy granulating surface. There is such a uniformity about the process that it may almost be said to be stereotyped. Contrast this with a case treated successfully by the more familiar antiseptic methods, even with the assistance of minor surgical operative treatment. In such an instance no definite time limit can be set to the cessation of the infective process. It may go on for days, or weeks or even months, as a glance at the temperature chart of almost any severe wound infection/

infection will show. In such cases the morbid process is essentially progressive in character, or at best is more or less protracted. In cases treated with the Reading bacillus, on the other hand, where this organism has been allowed freedom of contact with every part of the wound surface, one can practically foretell that in so many days the wound will be found perfectly clean. By the agency of the specific anaerobe the morbid process is brought rapidly to an end.

Having thus dealt at some length with one factor, namely operative interference, lest there be a tendency to give undue credit or to attribute to it the success which properly belongs to the biological treatment, there remain two others only which fall to be considered. One of these is the use of the salt bag, and the other the introduction of the Reading bacillus.

The former may be dismissed in a few words, as the cases and experiments described in the following pages furnish ample indication of the rôle they play. Even in wounds which have been most thoroughly explored and opened up, the subsequent packing with salt bags is not always followed by a successful result. Indeed it was with the very object of finding some explanation of such failures that the investigations/

investigations on which the thesis has been based, were undertaken by me. Some of these cases are complete failures, and in such it will generally be found that the wound behaved very much in the same manner as does a wound treated in the more familiar way with antiseptics, with or without occasional operative measures of a minor nature. Examples of such failures are given in the following pages.

On closer investigation a point of crucial importance emerges, namely that from these failures the Reading bacillus is absent, while as a corollary to this, I have found the Reading bacillus present in all salt bag cases which have proved successful here.

From a consideration of these facts it seems legitimate to argue that the sole factor necessary to ensure success is the presence in the wound of the Reading bacillus in a state of active growth. Conclusive evidence is furnished by some of the cases about to be recorded, where each of the possible claimants to the honour of being regarded as the sole agent by which the cure is effected, has been in turn eliminated, leaving only the Reading bacillus.

The wounds in this particular group of cases had been thoroughly dealt with surgically, and had then been packed with salt bags. No improvement after a due trial followed. Such ought to have taken place if/

if success depended either on the operative measures, the salt packs or a combination of the two. The specific anaerobe, however, was found to be absent on bacteriological investigation. It was then deliberately introduced in living culture and fresh salt bags inserted without further complicating measures. The result on this second occasion was complete success. The classical train of events followed, familiar to all who have had experience of the salt-bag method of treating wounds, the morbid process came to an end and healing ensued. The case for the Reading bacillus had been sustained.

That the salt is not the factor and indeed that it is even unnecessary is proved by the successful substitution, in some of the cases about to be recorded, of sphagnum moss and in one case, of simple sterile gauze, tightly packed into the wound after sowing with the specific anaerobe. These facts, as I have already indicated, merely help to furnish conditions favourable to the active proliferation of the essential agent, the bacillus which demands an anaerobic environment.

I. CASES illustrating unsuccessful treatment with salt bags where the Reading Bacillus was absent.

Case 1. Pte. D: admitted with a gun-shot wound of the left buttock and a smaller wound near the right anterior superior iliac spine, containing a foreign body. On arrival in Reading nine days after receipt of the injury, they were found to be suppurating and to have been plugged with gauze, but there was unfortunately no note of the precise treatment employed prior to his arrival here. For the next five days the patient ran an irregular temperature, ranging from 99° to 104°, with a pulse of 96 to 116. At the end of this time, as there was no apparent improvement, the wounds were thoroughly laid open by Captain Joyce, the foreign body removed and salt bags inserted. This, however, was not followed by a fall in temperature, and it was noted that the characteristic salt bag odour was absent. The salt packs were nevertheless left in situ for six days, but as there was no improvement, they were removed and Eusol dressings substituted. It was noted at the time of removal that the wound was oedematous and unhealthy, and that no separation of the sloughs had occurred.

Eusol dressings were persevered with for the next/

next twenty-one days before the wound could be pronounced clean and healthy, although the temperature came down after four days of Eusol treatment and remained down, with the exception of nine days, during which it fluctuated between 100° and normal.

Comments. This is an example of a septic wound packed with salt bags, which did not emit the characteristic "salt-bag" odour and which took forty days from receipt of the injury to reach a point where it could be considered a healthy granulating wound. In addition to the absence of the typical odour associated with the active presence of the Reading bacillus, the wounds after removal of the salt bags showed no separation of the sloughs and were still oedematous and unhealthy, affording ocular demonstration that a proteolytic organism had not been at work. The temperature had not fallen, suggesting in all probability that toxic substances were still being absorbed into the system. This case illustrates that the extraordinary improvement that follows the use of salt packs in a successful case can not be due either to the salt or to this method of treatment by "packs." It cannot be urged that the salt bags failed here for want of more radical surgical interference. The foreign body had been/

been removed and the wound freely laid open, and no further incisions were afterwards found necessary. These facts support the contention that the mere removal of a foreign body, the extraction of pieces of dead bone or the free opening up of a wound is not the factor which determines recovery and healing of that wound. In cases treated with salt packs which have developed the Reading bacillus and which have rapidly improved and become healthy, the fact that on the occasion of packing some foreign body or piece of bone was removed, has introduced a factor which adverse critics might conceivably regard as the real cause of the improvement. Here then is a case in point where thorough surgical measures were undertaken and at the same time salt packs inserted. Even this combination failed to alter the appearance or condition of the wound. The reason I venture to suggest is that no appreciable proteolysis occurred, and the patient continued to absorb toxic substances. The difference between this case and one that has succeeded under salt bag treatment is to be found in the presence in the latter of a proteolytic agent in the shape of the Reading bacillus.

In the case above described it ultimately healed although/

although somewhat slowly under the influence of Eusol, which itself appears to exercise among other things a proteolytic action.

Case 2. Pte. P.: was admitted to Reading War Hospital ten days after receipt of a gun-shot wound of the right knee, involving the joint. The wound, which was in a very sloughy condition, had been thoroughly exposed abroad, and the patella had evidently been removed. Six days after his arrival in Reading, the limb, above and below the joint, was noticed to be oedematous and a considerable amount of pus was expressed from under the skin on the inner side of the joint. Two days later the sinus was laid freely open by Captain Joyce. During the next five days the leg and thigh became still more oedematous, and the patient had a hectic look and temperature. The wound was at once thoroughly re-opened, multiple incisions made into the tissues around, and the whole well packed with salt bags. During the next five days, there was no characteristic odour from the wounds, the temperature still continued to jump, and the man was evidently going down-hill.

On the following day, after consultation, amputation was performed through the thigh, but the patient/

patient succumbed four days later.

Comment. This is another example of wound treatment by thorough opening up and free incisions followed by salt bag packing. No improvement whatever took place, the characteristic odour was absent, and the man died of septicaemia. The wound had been thoroughly exposed and explored before the patient's arrival in Reading. Further incisions were made without influencing the general condition of the man or modifying the spread of the wound infection. Yet another attempt was made by means of operative measures coupled with the use of salt bags, only to fail like the others. Again, neither operative interference nor the presence of salt bags in the wound, although both were here combined, served to ward off a fatal issue. Once more I would submit, the failure was due to the absence of a proteoclastic agent, able not only to attack the dead tissues, but also to prevent in some way the constant entry of toxic substances into the system. The presence or absence of such an agent is in my opinion the essential and determining factor between success and failure, and a consideration of the cases here recorded and the experimental work detailed further on will, I venture to think, substantiate my claim.

Case 3. Pte. J. D.: was admitted to the War Hospital, Reading, seven days after sustaining a gun-shot wound of the left arm.

On admission there was a through and through wound leading from the radial side of the flexor aspect to the ulnar side of the extensor surface of the forearm. The latter was much swollen. The wound was dirty and discharging freely, but there was no odour. There appeared to be no injury to the vessels or nerves.

He was operated upon, on the day of admission. The entrance and exit wounds were enlarged, and the track irrigated. A piece of cloth was removed and salt bags inserted. Cultures made previous to packing yielded a growth of streptococci but no anaerobe was obtained. The bags were left in situ for eight days, during which time the characteristic odour was not present. Cultures were again made on removal of the bags and streptococci obtained in pure culture. No anaerobe was found.

The patient ran a temperature all the time the bags were in situ, and continued to do so for some weeks later. The wound continued to discharge freely, but eventually healed up slowly under antiseptic/

antiseptic treatment.

Comments. This is an example of a recent gun-shot wound. Suitable surgical measures were early undertaken, including the removal of the foreign body present. Salt bags were introduced. If this were all that were necessary, one would have expected the wound to clear up in a few days, as is the rule in successful salt bag cases. Although the bags were left in situ for eight days, however, improvement did not begin. The typical odour was absent indicating that the organism if present was certainly not active. Cultures made both before and after treatment with the salt bags failed to reveal the presence of the Reading bacillus. From the point of view of the salt bag the case was a failure, because the one factor essential to success was absent. The case, however, lends point to my argument that efficient operative measures by themselves or even in conjunction with salt packing are not followed by the rapid improvement in the patient's general condition or the speedy healing of the wound where the Reading bacillus is absent from that wound.

II. CASES illustrating successful salt bag treatment where the Reading bacillus developed without requiring to be sown, but where in addition some minor operative measures were taken.

Case 4. Pte. L.: was admitted to Reading with a gun-shot wound of the right leg together with a compound fracture of the femur situated about 4" below the great trochanter received about three months previously. He looked anaemic and ill. The history was as follows. - The patient lay out in the open for four days before being brought in, and he remained at the Casualty Clearing Station for eleven days, during which time gas gangrene developed, and he became very ill. He was then removed to a base hospital, where the wounds were explored and drained. No notes of the type of dressing employed at this time are available. The fracture was treated at first on an abduction frame and later, on a Hey-Groves splint. There was 1" of shortening and the position of the bones was bad. A sequestrum was removed at a subsequent operation after/

after which a Wallis extension was used. The wound was next packed with salt bags, but for some unknown reason, these were removed on the following day, his temperature being then 102.4° and his pulse 138. The wound was then syringed with hydrogen peroxide. His clinical condition, however, still remained bad. He was later transferred to England, on an abduction frame, and for the first few days after his admission to the Reading War Hospital, he appeared to make satisfactory progress. His temperature during the next seven days, however, began to rise, and the thigh became swollen and oedematous. The patient was thereupon anaesthetised, the wounds opened up and the bone drilled. The latter was found to be soft and infiltrated with inflammatory products, but no free pus was discovered. The wounds were then packed with salt bags. Two days later, the wounds were emitting a powerful and characteristic odour and the edges looked dirty and sloughy. After remaining in situ for six days, the salt bags were removed, and the patient's condition was excellent. Cultures were made from the pus, and the specific spore-bearing anaerobe to be described later was recovered, together with pyogenic organisms.

Normal/

Normal saline dressings were then substituted, and in three days' time the wound was found to be clean, and lined with healthy red granulations. A few days later a small sequestrum came away in the dressings, and in six weeks' time from the introduction of the salt bags, the wound was completely healed.

Comments. This case illustrates several points. The patient had suffered considerably from exposure and had developed gas gangrene. He had apparently been tided over this acute attack, but the wound was still septic and became worse after his arrival in Reading. Salt bags had been employed for one day only abroad, but after his arrival here they were re-introduced and left in situ for some days. The temperature fell the day following the introduction of the salt packs and remained normal thereafter. The wound, judging from the odour which developed, contained the specific anaerobe, and this was proved by its recovery from the wound. The extraordinary and rapid improvement which here followed the salt pack treatment might altogether or in part be claimed by some as due to the operative measures undertaken immediately before the salt bags were inserted. The wound, however, had been thoroughly opened up before and explored, and a little later a sequestrum had been/

been removed. This, however, had not influenced his condition to any appreciable extent beyond apparently stopping the progress of the gas infection. When, however, salt packs were introduced, rapid improvement followed. The patient's general condition quickly became altered for the better, in my opinion because the absorption of toxic substances was in some way interfered with. This could not be due to improved drainage, for with salt packs the drainage is probably not as efficient as in some other methods of wound treatment. It could not be due to the fact that a septic focus had been removed surgically at the time of operation, for no excision was attempted. It could not well be due to the salt bags per se, for wounds so treated which fail to develop the characteristic odour indicative of the presence of the Reading bacillus do not, in my experience, improve. Free incisions certainly had been made, but free incisions do not remove the infection. Yet this case improved and that rapidly. Why? My answer is that there was present the protocl^eastic agent, the signal of whose active presence was the development of the characteristic odour, the visible evidence of its activities, the healthy/

healthy condition of the wound after removal of the packs and the actual proof of its identity, the recovery of it in culture.

Case 5. Pte. W.: admitted to the Reading War Hospital suffering from gun-shot wounds of both shoulders received twenty-one days previously. There was a large wound over the right scapula. The exposed part of this wound showed healthy red granulations. Overlapping the wound was a flap of skin covering a portion of the wound which was very dirty and full of foul pus. The fractured scapula was partly exposed. There was a counter-incision over the supraspinous fossa communicating with the large wound. Over the spine of the left scapula was a clean, superficial wound. Carrel-Dakin treatment had been begun the day following receipt of the injury and this treatment had been persisted in till he was sent over to England, the tubes being still in situ. On arrival here, cultures were made, from which I was able to isolate staphylococcus aureus, a streptococcus brevis, a Gram-negative bacillus, and in addition the specific anaerobe which I have come to associate with successful cases. On arrival in Reading Eusol//

Eusol treatment was begun, and the wound began to look cleaner and the tissues to be less swollen. Eighteen days later, as pus had again begun to accumulate, and as during the whole of that period the temperature had kept fluctuating, the wound was opened up and several foreign bodies and pieces of bone were removed, after which it was packed with salt bags.

In three days' time, the wound was emitting a powerful and characteristic odour, and the temperature had come down. When the bags were removed after having been in situ for seven days, the wound appeared perfectly clean and healthy.

Comments. In this case the specific anaerobe was certainly present when the case arrived in Reading. Without excision of the wound, Carrel-Dakin treatment had been carried out probably for eighteen or nineteen days after receipt of the injury. Nevertheless the charts for this period showed an up and down temperature, and one at least of the wounds looked unhealthy. During a further period of eighteen days' treatment with Eusol, no improvement in the temperature took place. As soon as the wounds were opened up, however, and salt bag packing used/

used, improvement took place and was maintained.

Presumably the specific anaerobe was early present in the wound, along with other organisms, but as the conditions favouring its growth and activity were absent, improvement did not occur either under the Carrel-Dakin or under Eusol treatment.

As soon, however, as the conditions were rendered favourable, the organism became active and the wound rapidly cleared up.

Here again it may be argued that the improvement that followed the insertion of the salt packs was due to the removal of foreign bodies and fragments of bone. One cannot in this case state definitely that the surgical interference did not contribute to the improvement in the wound. I can only point to the fact that the temperature only came down after three days of packing, that this fall synchronised with a vigorous development of the Reading bacillus, and that in a comparatively few days the wound was clean and healthy and gave no further trouble. On the other hand many instances are cited in this thesis, and every surgeon must have encountered such, where thorough removal of bone fragments and foreign bodies was not followed by improvement, or where improvement did occur, the change was not a rapid and dramatic one as in the case just mentioned, for the/

the clinical course of cases successfully treated with salt bags is in a class by itself.

Nor could the improvement be due to the presence of salt packs by themselves, for not a few salt packed cases fail, but those which fail are devoid of the characteristic odour and the Reading bacillus is absent from them.

It is certainly fortunate for the salt bag method of treatment that the specific organism appears to be so often naturally present, otherwise I presume there would be many more cases of failure with this method of treatment.

Case 6. Pte. F.: was admitted to the Reading War Hospital suffering from an accidental gun-shot wound of the left thigh, testicle, and right arm, received the same day, several foreign bodies being present in the wounds. There was an entrance wound just below the fold of the left buttock and a large explosive exit wound situated in the upper third of the left thigh, a wound of the left side of the scrotum and a transverse wound on the flexor aspect of the right wrist. In the region of the thigh wound, there was much destruction of muscle tissue. The femoral artery was exposed and lying bare on the outer side of the wound. A piece of the tuber ischii had/

had been chipped off and a channel passed up into the pelvis.

Lister's technique was used by my colleague for disinfection of the skin. The wound was irrigated with 1 in 3,000 biniodide of mercury, and salt bags were introduced. The testicle was completely removed and the wound in the wrist stitched up. In a couple of days, the wound in the thigh began to emit the characteristic odour which is associated with the activities of the specific anaerobe. After four days, however, as the temperature was steadily rising instead of falling, as it ought to do in such a case, the salt bags were removed. The characteristic odour was now intense. The edges of the wound looked black, sloughy and semi-digested, but there was no swelling, no oedema and no redness anywhere near the wound. From cultures made from the pus I was able to recover the specific anaerobe together with staphylococci and diphtheroids. Eusol irrigation was substituted for the salt bags and next day it was noted that the dead muscle was coming away freely in fairly large quantity. The wound still presented a greyish-black appearance and still smelt.

The temperature, however, had not come down, and, on further investigation, it was found that the right hand/

hand was swollen and oedematous. The stitches were accordingly removed from the wound in the wrist, when a considerable quantity of pus escaped. The characteristic smell was absent from this wound. Three days after removal of the salt bags, the wound in the thigh was found lined by healthy red granulations with, here and there, a few specks of sloughy material on the surface and a larger slough on the outer side of the wound. There was some brown foul smelling pus in the wound, but no surrounding oedema or erythema. Eight days after removal of the salt packs, the wound presented a brilliant red colour and looked exactly like a text-book illustration of a dissection of Scarpa's triangle. The temperature did not come down till the wound in the wrist began to improve.

Comments. This case also affords material for consideration. In the first place, it is practically the only case, out of a large number of gun-shot wounds received in Reading, to be seen immediately after infliction, and, like all gun-shot wounds, there was extensive destruction of muscle. As I have already stated, the observations made in the previous pages and the method of treatment therein advocated, deal almost entirely with septic wounds several days or even months old before they are seen here. In this particular instance, the wounds were seen/

seen and treated within an hour or two of receipt of the injury.

From the fact that the specific anaerobe made its presence known in one of the wounds within the first few days, and was isolated in culture, we may fairly conclude that it or its spore form was implanted practically at the time of the accident. The same patient, however, had a control wound in which, to judge from the absence of odour, the specific anaerobe was not present. This wound was apparently of so slight a nature that it was resolved to stitch it up at the time. When the temperature did not begin to fall as it was expected to do, my colleague became alarmed and removed the salt bags, under the impression that it was the severer wound of the two that was going wrong. This, in the light of further experience and on more mature consideration, was a mistake. This wound, in spite of its extent and severity, was making satisfactory progress towards recovery, and, although sloughy and black, showed no evidence of acute infection. The rise of temperature was in reality due to the progressive infection of the wrist wound. The power of the latter for evil was not sufficiently realised in view of the fact that it was slight, was seen almost immediately after infliction/

infliction, and had been well treated by the old antiseptic method. The case is of value, inasmuch as it helps to illustrate what I have enunciated further back, viz: that when a wound, packed with salt or sphagnum moss bags, is comfortable and is giving forth the characteristic odour and yet the patient's temperature has not come down, it is time to consider whether there is not some focus elsewhere which has been missed either inadvertently, or because of its apparently trivial character. In the present case the dead tissues separated fairly rapidly, but probably not so rapidly as they would have done if the packing had been left undisturbed for some days longer. The devitalised tissues were obviously in process of being digested, and this particular wound was in a healing condition, before the wrist wound, which had not been treated by the salt pack method, had begun to clear up.

This case is also instructive inasmuch as it indicates that if the Reading bacillus is used sufficiently early in cases with extensive damage to the soft tissues, it acts just as well as in cases seen at a later date, and that it would probably save much long continued suppuration and obviate a good deal of operative manipulation consequent on that suppuration.

Case 7. Pte. R. A.: admitted to Reading War Hospital fifty-two days after receiving gun-shot wounds of right hip, right arm and right hand. Previous to his arrival here the right leg had been amputated below the knee. The flaps were fairly clean and the amputation stump gave no further trouble. In the region of the right buttock there were two large and several small dirty wounds. Previous to his arrival several incisions had been made into the right upper arm, which was somewhat wasted, but showed no oedema. These arm wounds eventually healed up without incident. There was a through and through wound of the right wrist, and two wounds in the hand. The palm of the hand was swollen and the flexor tendons in a state of contracture. The wrist wounds were treated in an arm bath, and the other wounds with Eusol.

Five days later considerable pocketing of pus was found in relation to the wounds in the wrist and in the right buttock. The patient was also still suffering from the shock sustained by the torpedoing of the ship in which he was being brought to this country.

Nineteen days after his arrival here the man was anaesthetised and the thigh wounds explored. An abscess/

abscess was found passing down the outer side of the thigh. The pus was evacuated, the cavity laid completely open, and packed tightly with plain sterile gauze. The walls of the cavity were loosely approximated by the insertion of three salmon gut sutures. From cultures made at the time of operation, *Staphylococcus Aureus*, *Streptococcus Longus* in large numbers and the Reading bacillus were isolated. The packing was left in for five days during which time the patient felt considerably better, but while the temperature had fallen, it had not come down completely. The wound emitted the characteristic odour, and the Reading bacillus was recovered in culture.

The three stitches were now removed as well as the packing, when it was found that the wall of the cavity was clean and lined with healthy red granulations. In twenty days from the time the packing was removed the wound in the thigh was represented merely by a line of clean healthy granulations.

During this time, however, the wounds in the wrist and arm were being treated with Eusol. A month after admission the hand was still swollen although/

although less so, while the wound in the wrist was fairly clean.

Fifty-two days from the date of admission the hand and wrist again became swollen, oedematous and very painful. This was accompanied by a rise of temperature.

Operative interference was deemed advisable and the wounds in the wrist and hand were thoroughly reopened and packed with salt bags. These were left in situ for four days during which time the hand still remained painful. No characteristic odour was apparent, the temperature had not fallen and the pulse-rate had not diminished. The bags were removed and it was seen that the wound surfaces were not lined with healthy granulations. The latter looked pale and sodden. Eusol irrigation was substituted for the salt bags and under this treatment the hand and wrist wounds gradually cleared up.

Comments. This case illustrates several points raised in this thesis. In the first place the man sustained multiple wounds and it had been found necessary to amputate his right leg. Of all these wounds only those in two places continued to give trouble, viz. those in the buttock and those of the wrist/

wrist. The former had been treated abroad for fifty-two days, but the nature of the treatment was not ascertainable. For nineteen days after his arrival in Reading Eusol was employed. So far from overcoming the infection, the latter appeared still to be making headway, as witness the tracking of an abscess along the thigh. This was evacuated and the parts laid open. The Reading bacillus was present along with streptococci, etc. as was proved by culture but the environment had not been suitable for its development. As soon as this was rectified, the specific anaerobe was able to proliferate, and its presence was speedily advertised in the usual way. It is noteworthy here that instead of salt or sphagnum moss packs, plain white sterile gauze was used for packing. This was tightly packed in, and when soaked in discharge formed an efficient anaerobic plug. It indicates that there is no special virtue in the salt, as indeed the successful substitution of sphagnum moss had already shown. Hence in a case where nothing has been done beyond sowing and packing (and I cite cases of this kind,) the improvement must be associated with the activities of the Reading bacillus and with that alone. In this case, it is true an abscess was opened/

opened. But the very fact that there was an abscess there after ten weeks of treatment by other methods rather goes to prove the comparative inefficacy of these particular modes of treatment. The fact emerges that the septic process was progressive under the methods of treatment adopted, but when the Reading bacillus was given its chance, the wounds in five days had become healthy, clean, granulating surfaces, and in twenty days they were practically healed! The case, however, illustrates something more. Although there was a definite improvement in the patient's general condition, it was not absolute as is usual in straightforward cases treated with the Reading bacillus. The temperature did not fall completely. The reason of course lay in the fact that the wounds in the wrist were still in a state of sepsis. They had at first been treated in an arm bath. Later Eusol was substituted for this form of treatment, and, after using this for at least three weeks, the wounds in the wrist became fairly clean, but the hand still remained somewhat swollen. Later instead of continuing to improve, a recrudescence of the infection occurred. The hand and wrist again became/

became swollen and painful, and there was an increase in temperature. The wounds were enlarged and packed with salt bags. These were left in situ for four days, but there was no sign of the Reading bacillus being present. The characteristic odour was absent, the pulse rate had not diminished, the temperature had not fallen, and on removal of the bags healthy granulations were not present. Instead of now sowing the wounds in the wrist with the Reading bacillus, recourse was again had to Eusol. The wounds ultimately cleared up under this treatment, but not in the rapid manner which is characteristic of the Reading bacillus. In this one case we have an example of a wound containing the Reading bacillus which did not heal up till the conditions were rendered favourable for its growth. No salt bag was used for this purpose. Another wound was actually packed with salt bags, but remained uninfluenced by this treatment, because the Reading bacillus on which, in my opinion, the whole success of the method depends, was absent.

III. CASES illustrating successful treatment by
the introduction to the wounds of the
Reading bacillus coupled at the same time
with surgical interference of a minor
character.

Case 8. Pte. S.: was admitted to the Reading War Hospital suffering from a gun-shot wound of the left foot and right leg received eight days previously.

Prior to his arrival, pieces of shrapnel had been removed from both wounds. The inner side of the left foot showed a small suppurating wound situated just above the base of the first metatarsal bone. The outer side of the foot was oedematous and there were through and through wounds, about two inches apart, situated about the middle of the fibula. Three days later the wound in the foot was opened up for the purpose of extracting a foreign body, and the resulting cavity was then packed with Morrison's "Bipp." Nine days later he was sent to an auxiliary hospital. A small piece of bone came away nine days after leaving Reading. Five days after this the patient complained of severe pain in the foot, and/

and he had a temperature of 100° . Fomentations were ordered by the doctor in charge, but as the foot became more swollen and painful during the next two days, he made an incision on the inner side. Pus was already freely discharging from the outer side. The temperature, however, was 101.4° , and recourse was had to saline baths and fomentations. Seven days later, as there was no improvement, the patient was anaesthetised and several pieces of the scaphoid bone were removed. Multiple incisions were then made, pus evacuated and drainage tubes inserted. Next day the foot was still greatly swollen, discharge was scanty, and the temperature continued high. As there was no appreciable improvement the patient was sent back three days later to the central hospital for "further treatment or amputation." On arrival again in Reading, the patient was anaesthetised, and, after taking samples of the pus for purposes of culture, multiple incisions were made by my colleague into the foot, when it was seen that the mid-tarsal joint was involved. The wounds were then irrigated with hot normal saline, after which I freely sowed both the old wounds and the newly-made incisions with a living culture of the specific anaerobe/

anaerobe. The wounds were then packed with salt bags. From the cultures taken before sowing, I recovered a heavy growth of *streptococcus longus*, together with a few colonies of *staphylococcus aureus* and *albus*. No anaerobe was isolated at this time. The temperature soon began to fall, and the characteristic odour to make its presence known. The salt bags were removed after having remained in situ five days, and the wounds were then irrigated with normal saline solution. They were found to be lined with a sero-purulent exudation and there were some dirty sloughs, which, however, cleaned up at once under the irrigation. Cultures were made from the wounds before irrigating, and again I was able to isolate *streptococcus longus* and mixed *staphylococci*. The total number of colonies was as large as on the first occasion of examination, but the relative proportion had altered, the colonies of *streptococci* being less numerous. Further, on this occasion I was now able to recover the specific anaerobe. Three days later, under simple normal saline dressings, the remaining sloughs were found to be fast disappearing and the wounds were becoming cleaner. Cultures were again made at this point, and the bacterial flora were now found/

found to consist mainly of mixed staphylococci, diphtheroid bacilli and a very few streptococci.

~~Three~~ Three days later the wounds were perfectly clean, and lined by firm, red, healthy granulations. In cultures taken at this point, I found only a few colonies of staphylococci and diphtheroid bacilli, but was unable to recover either streptococci or the specific bacillus.

In about six weeks from the date of removal of the salt bags, all the wounds in the foot were healed, the movements of the foot were much improved, and there was considerably less talipes equino-varus.

Comments. This case appeared to be doing well under "Bipp" treatment after surgical measures had been taken. In spite of this, however, there must have been a latent infection which subsequently flared up, and this is certainly not the only case I have seen where infection flared up again while the wound was still under the influence of B.I.P.P. In spite of multiple incisions, saline baths and fomentations, the foot went from bad to worse. The patient became extremely ill, the foot greatly swollen and bright red and the discharge scanty. Bacteriologically, the predominant infection present was a streptococcus longus/

longus.

After making some fresh incisions into the soft parts, copiously sowing the wounds with a living culture of the specific anaerobe and packing with salt bags, the condition of the patient at once altered for the better, and the foot made an uninterrupted recovery. The "Bipp" treatment had not in this case been an unqualified success, and the ordinary methods of treatment which had been adopted proved unavailing. It might again be urged against the claim of a proteolytic agent to be considered causal that the improvement which followed the use of the salt bags was due, not to the activities of an organism induced to grow under suitable conditions, but to the fact that the experiment, if so it could be called, was complicated by operative measures. These operative measures, however, were confined to the making of a few fresh incisions.

Further, during a period of something like six weeks, at least four operations had been performed, all of them involving incisions into the tissues, and two of them undertaken for the removal of foreign bodies and fragments of bone. None of these had been followed by improvement. Two or three days after the bags had been inserted, however, the temperature/

temperature began to come down and the patient to improve. A few days after removal of the salt bags the wounds were clean and healing. It seems too much to expect that a few incisions into the soft parts of a foot, the seat of an acute and virulent streptococcal infection, should be able within a day or two, without anything other than the presence of salt in the wounds, to stop the advance of the streptococcal infection. It would be all the more extraordinary in view of the fact that large numbers of streptococci were still present. Previous incisions and then operative procedure had failed to achieve this result. So far indeed were they from checking the infection that it was becoming ever more widespread. The patient showed constitutional symptoms indicative of a septicaemic condition. Something, within two or three days after the insertion of salt bags, put a stop to the further absorption of toxic substances. That it was the result merely of making fresh incisions seems sufficiently discounted from a study of this and other cases here recorded. That it was not the salt seems equally indicated from a consideration of cases that have failed under salt bag treatment and from the fact that a simple substance like sphagnum moss can be substituted/

substituted for the salt.

There is one other factor on which alone, in my opinion, hinged the success of the case. This factor was the Reading bacillus, found only in an active condition in cases doing well, absent in cases not doing well, although such had previously undergone thorough surgical treatment.

Owing to the length of time that streptococci remained about the wounds after treatment with the salt bags, one is entitled to question the efficacy of salt, used in this concentrated form, as a means of cleansing the wound of pathogenic organisms. It is interesting to note in this connection that Tuffier,¹⁹ speaking at a meeting of the Academie de Medecine of Paris, stated that Wright's hypertonic saline solutions were powerless against the streptococcus.

At the same time, it seems obvious from this and other cases, that the specific anaerobe, when present, does not produce its beneficial action by directly causing the disappearance of the infecting pathogenic organisms through symbiosis with them, since they may be found about the granulation tissue for some days after removal of the salt packs. This view is supported by/

by the experimental work discussed further on in this thesis. The Reading bacillus effects its good results not by overgrowing an organism or organisms in symbiosis with it, nor yet by producing some body which inhibits the growth of these other organisms. It does not appear to act as an antiseptic is supposed to do, by direct attack on the pathogenic organisms themselves. Its action is essentially a proteoclastic one whereby its enzymes break down into non-toxic substances the dead tissue, and at the same time appear to attack in the same way tox-albumens formed by other pathogenic organisms. This is discussed, however, elsewhere.

Case 9. Pte. S-d.: was admitted to the Reading War Hospital with a gun-shot wound of the left thigh and leg inflicted about two months previously. The history of the case before arrival in England was as follows. There had developed, after receipt of the injuries, a very severe gaseous cellulitis of the leg and a less severe one of the thigh. Carrel-Dakin treatment had been used throughout, and the patient had already been in the operating theatre on twenty-five occasions for various operative measures before being sent to England. On his arrival here the limb was/

was still very oedematous, the four leg wounds looked unhealthy, and the granulations were sodden and oedematous. The latter were traversed by tunnels which in turn were occupied by Carrel-Dakin tubes.

The wound in the thigh presented a similar appearance to that in the leg, and was discharging pus in considerable quantity.

The patient was anaesthetised, and all five wounds were laid freely open by my colleague, Captain Joyce. Deep collections of pus were evacuated from each, and several small sequestra removed. The wounds were thereupon swabbed out with plain dry gauze, after which I introduced the specific anaerobe in living culture. Each wound was then firmly packed with salt bags moistened in sterile physiological saline, and layers of gauze similarly moistened were laid across the packed wounds. Cultures which I made before sowing yielded a growth of bacillus pyocyaneus and a diphtheroid.

The temperature rose after this procedure, as it does in most cases so treated, but the patient felt comfortable, and in two days' time, the oedema of the limb had commenced to diminish. The salt bags were left in situ for nine days, during which time the characteristic odour made its presence strongly felt. On removal/

removal of the packing, the superficial layers of gauze were found to be dry, brownish in colour, board-like in consistency and firmly adherent to the skin all round the wound. After loosening the adherent edges by means of gentle traction, the whole packing came out easily en bloc, completely bathed in yellow pus. The specific anaerobe was now recovered, but *b. pyocyaneus* and another Gram-negative bacillus were still present. The wound of the thigh was then irrigated with Eusol to clear away the discharges, after which it was seen to be perfectly clean and red. Part of the fractured bone could be seen at the bottom of the wound, looking white and clean. All oedema had disappeared from the tissues around. The wounds in the leg presented a similar appearance, but there were still present in them a few thin delicate sloughs which looked as if they were nearly digested, while bright red healthy granulations could be seen covering the raw surfaces. All oedema had disappeared from the ankle and foot and most of the swelling from the leg. The wounds were simply irrigated with Eusol, dressed with plain gauze daily and healed up without any further trouble.

Comments. This case presents several interesting features. To start with, there were multiple severe wounds/

wounds with comminuted fractures both of the femur and of the bones of the leg, and, to add to the seriousness of the case, these were found to be infected with gas-producing organisms. It was an excellent case on which to test the efficacy of the Carrel-Dakin method of treatment, which was persevered with for the two months prior to his arrival in England. During this period this treatment was supplemented by no less than twenty-five operations of one sort or another. It is true that not only was the man's life saved, but also his limb. After two months of this treatment, however, the wounds were still unhealthy, and full of pus, while the limb was greatly swollen and oedematous. The original gas-infection had been combated, but the Carrel-Dakin treatment had failed to rid the wounds of their aerobic infection. This, I am told, is not infrequently found in cases treated solely by the Carrel-Dakin method. It stands out in marked contrast with one or two wounds which I have seen recently, stated to be examples of the Carrel-Dakin treatment. These latter were beautifully clean, with a typical raw-beef colour and well covered with healthy granulations. On examining these wounds more carefully, it was plainly evident that the original/

original wound had been excised, and that what I saw was the excised wound which had subsequently been treated by the Carrel-Dakin method. The success in this recent group of cases should not legitimately be claimed by the advocates of the Carrel-Dakin method. I should like, therefore, in estimating the value of a given method of treatment, to draw a sharp line of demarcation between those cases treated by the Carrel-Dakin method alone, and those which may be regarded as samples of excision followed by the Carrel-Dakin procedure. This latter is quite a different thing from the former, and has no right whatever to be spoken of as the Carrel-Dakin method.

In vivid contrast with the clinical course of the case during its previous two months of Carrel-Dakin treatment, stands out its subsequent progress after the application here of what I call the biological method. Without further operative measures beyond re-opening up the wounds, permitting pus and imprisoned discharges to escape and removing a few small sequestra, the wounds were well sprinkled with a living culture of the specific anaerobe and then packed in the way described.

Within ten days from the commencement of this treatment, the wounds were healthy, covered with strong red/

red granulations and all oedema had disappeared! Here again it may be urged that it was the removal of the small sequestra that brought about the alteration in the patient's condition. The removal of small fragments of bone as being sufficient in itself to account for the speedy recovery of this and other cases, I have already discussed.

It is certainly extraordinary that after no less than twenty-five minor operations for the removal of foreign bodies, bits of bone, etc. performed on various occasions over a period of two months, coupled with prolonged Carrel-Dakin treatment, the limb was still oedematous, contained collections of pus and the wounds septic and dirty.

It would be still more extraordinary if the removal of a few fragments of loose bone should, in Reading, be followed by rapid and complete recovery. One might venture to suggest that the small sequestra removed here were in point of actual fact the result of the prolonged infection, and that if the former treatment had been persisted with, the twenty-sixth operation would have had to be followed by others for the further removal of bone due to the continued ravages of septic organisms.

The clinical course of this case after treating it/

it with the Reading bacillus was however different from that with which one is familiar, as a sequel of operative interference followed by antiseptic treatment. It is, however, on all fours with the clinical history of all cases treated with salt or with sphagnum moss bags where the Reading bacillus was already present or had been implanted by the bacteriologist.

Case X. Pte. H-n.: received a gun-shot wound of the right thigh eight weeks prior to his admission to the Reading War Hospital. The femur was fractured at the same time.

According to the notes received from France, there was a ragged wound on the inner side of the knee to the left of the patella. There was a compound fracture of the bone in the internal supra-condylar region and near the adductor tubercle. There was also a gutter fracture between the condyles. A foreign body was removed on the same day as the injury was sustained. The wound was excised, cleansed, irrigated and B.I.P.P. smeared over the raw surfaces. The synovial membrane was then stitched up. From the same notes it appears that there/

there was a considerable amount of subsequent suppuration and that Carrel's tubes were inserted for treatment by the Carrel-Dakin method. Twenty-two days from receipt of the injuries a partial secondary suture was attempted. On admission to the War Hospital, Reading, eight weeks after receipt of the wounds, the following was the condition of the parts.

There was a large square granulating wound in front of the left knee. The patella had apparently been removed previously. The granulations were pale and oedematous and tunnels, occupied by Carrel's tubes, ran in towards the centre of the limb. There was a large fluctuating swelling in the popliteal space and the thigh muscles were markedly wasted.

Six days after admission to the Reading War Hospital, the wounds were reopened and an abscess evacuated from the popliteal space.

The wounds were then sown with the Reading anaerobe and packed with salt bags. The pus contained large numbers of streptococci. The characteristic foul odour soon became noticeable and the patient's general condition began to improve. Six days later the packing was removed. The latter was/

was found as usual saturated with thick pus. After irrigation the wound surfaces were seen to be covered with healthy red granulations with some thin sloughs partly adherent. The swelling of the leg and thigh had largely disappeared. Ten days later the knee presented quite a different aspect from what it did prior to treatment with the Reading bacillus. The wound was everywhere lined with healthy, firm, red granulations. All swelling of the limb had disappeared and the temperature had remained normal or subnormal. The pulse, which before treatment with the Reading bacillus varied from 120 to 100 per minute, fell four days after sowing with the bacillus to 96, and subsequently ranged from 70 to 80. The wounds healed up completely without further interference or trouble, except for a small sinus which persisted for some time afterwards.

Comments. This case was one of severe injury to the knee joint. It received very radical treatment on the same day as the wounds were sustained. A foreign body had been removed, and the wound excised, cleaned and treated according to the Morrison technique. In spite of this radical treatment, the case was a failure so far as excision, B.I.P.P. and re-suturing were concerned. Suppuration followed and recourse was/

was had to the Carrel-Dakin method of treatment. This was equally a failure as is evidenced by the condition of the limb on arrival in Reading, after the Carrel-Dakin treatment had been persisted in for over a month. In spite of that treatment, the wound was oedematous and unhealthy, the limb was swollen, and an abscess had formed. In other words, in the face of that treatment, the infection was progressive as is proved by the formation of an abscess. Streptococci were present in large numbers. The man had a rapid pulse and was running a temperature which daily reached 102° or 103° F. He was distinctly ill. Both Morrison's B.I.P.P. and Carrel-Dakin's technique had apparently failed. Without further interference, except for evacuating the pus and opening up the wounds sufficiently to allow of proper packing, the wounds were sown with the Reading bacillus. Within eight days from this point, all swelling and oedema had gone, the wound was perfectly healthy, and covered with firm healthy granulations. The patient's temperature and pulse became normal and the wounds healed up without further trouble. The progress of the infection had been brought to an end practically within the short/

short period of eight days after sowing with the Reading bacillus, while nearly nine weeks of anti-septic treatment prior to this had failed to limit the infective process.

IV. CASES illustrating successful treatment
solely by the introduction to the wounds
of the Reading bacillus unaccompanied by
operative measures.

Case 11. Pte. G.: was admitted to Reading with a gun-shot wound received eight days previously. There was a large ragged wound on the posterior and outer part of the right calf. Some of the muscles and tendons were sloughing. Fusol and normal saline dressings were being used. The patient's temperature fluctuated between 99° and 104°. The infection, in spite of this treatment, was apparently progressive. Pus had accumulated towards the outer side of the leg, apparently burrowing along the tendons of the peronei muscles, which had already partially sloughed. From cultures made, I was able to demonstrate the presence of streptococci, staphylococci, and a Gram-negative cocco-bacillus, but failed to find the Reading bacillus. The wound was then laid freely open, after which I sowed it with a living culture of the specific anaerobe and it was then packed in the usual way with salt bags. The wound began to smell strongly, and on the fourth day the temperature came down and remained normal thereafter.

Seven/

Seven days later the salt bags were removed, and, after irrigation with Eusol, the wound was found to be quite clean and lined with healthy granulations. The specific anaerobe was recovered from the wound together with *B. proteus vulgaris*, but streptococci and staphylococci appeared to be absent. Seven days later the wound was ready for skin grafting.

Comments. Treatment by Eusol and saline while they were being employed, did not appear to be able to limit the activities of the pathogenic organisms present. Pus was still accumulating and the tissues were still in a sloughing condition.

At this point the wound was sown with the Reading bacillus. In this particular instance the operative procedure was confined to enlarging the wound. No pieces of bone and no foreign bodies required removal, so that the subsequent improvement could not be attributed to the removal of such. Yet this wound behaved exactly like others treated with salt bags where the Reading bacillus was definitely present and active. The temperature came down on the fourth day, remained thereafter normal, and the wound was speedily in a condition when skin-grafting could be undertaken.

The/

The clinical history of the case subsequent to sowing with the Reading bacillus is on the other hand quite different from that which is observed either after simple opening of a wound followed by anti-septic treatment, or after the use of salt bags where the specific anaerobe is absent.

Case 12. Pte. C-: was admitted to Reading War Hospital, with a compound fracture of the femur received in France nineteen days previously. A week after his arrival in Reading the wound was opened up thoroughly, cleansed and B.I.P.P. applied according to Morrison's technique. Ten days later it was noticed that the wound looked oedematous, and unhealthy while the whole thigh appeared to be swollen.

The wound was well opened up and various other antiseptics tried during the next six weeks. At the end of this time, as the wound failed to respond to the various methods of treatment employed, and the thigh still remained swollen and oedematous, the wounds were packed with salt bags. The day following the temperature rose slightly higher than it had been before, but by the sixth day it had come down to normal and never rose again. The typical odour was strongly in evidence and on removal of the salt bags, the/

the wounds, after irrigating with sterile saline, were found lined by the usual healthy red granulations. A few thin shreds of slough on the point of separation were all that was left of the dead tissue. The wound rapidly filled up and healed. About three months later, the patient was anaesthetised, and four large sequestra were removed. These sequestra were beautifully clean and ivory like, and after rinsing under the tap were seen to be devoid of all soft tissues. The removal of the sequestra was not followed by any flare up of the temperature. The fracture showed sound and firm union.

Comments. This case arrived in Reading nearly three weeks after infliction of the wound. The latter was septic and sloughing, and the femur had been smashed. The patient was running a temperature.

The wound was more thoroughly opened up, explored and cleansed. Treatment with B.I.P.P. by Morrison's technique was tried, but apparently failed. In the same way, treatment by other antiseptics proved unsatisfactory, although ample time was given for them to act. The temperature still continued to swing daily, the wounds did not appear to be improving, and the/

the thigh remained swollen. Without further interference on the part of the surgeon, either in the shape of removing dead bone, or of making fresh incisions, salt bags were packed into the wound. In a few days the patient began to improve. On the sixth day the temperature was normal, and within a day or two of removal of the salt bags the wounds were in a healing condition and all swelling and oedema had gone. While the salt bags were in situ the wounds smelt strongly of the Reading bacillus, which, owing to this method of dressing, had been enabled to proliferate and to carry out its proteolytic functions.

It is noteworthy also that several sequestra which eventually became loose and were removed, gave rise to no trouble while they were in process of separation, and their removal by operation was without any untoward effect on the patient. The sequestra were devoid of soft tissue. Nothing was done at the time of packing to which hostile critics might attribute the improvement, beyond the insertion of the salt bags. The improvement began within three or four days of packing and synchronised with the proliferation of the proteolytic organism.

Not/

Not only was nothing done in the way of removal of loose fragments of bone on the occasion of packing, but after recovery had taken place, fragments of dead bone remained for some considerable time in the limb without giving rise to any untoward symptoms, and they were subsequently removed without lighting up any further septic trouble.

On the other hand I have cited cases to show that thorough opening up of a wound and the removal of all fragments of loose bone are not necessarily followed by subsidence of the infective processes with consequent healing of the wound. Apart from foreign bodies and fragments of dead bone in a wound, there are other factors viz. devitalised or dead soft tissues and the pathogenic organisms which populate them. These are the factors which it is important to get rid of since it is by the action of these organisms on the dead tissue that substances of a toxic nature are elaborated which not only poison the patient but produce further local death of the tissues so that the process may go on without a check.

Case 13. Pte. J. B.: was admitted to the Royal Berkshire Hospital, Reading under Major Guilding suffering from multiple shrapnel wounds of the right leg, received twenty days previously.

The notes received from France stated that there were multiple dirty wounds of the right leg. There was a comminuted fracture of the tibia and fibula near their upper extremities, and a gas infection had supervened. Four days after receipt of the injury a foreign body had been removed and wide incisions made. There is no record of the specific treatment employed in France. On admission to the Royal Berks Hospital, there was found a large healthy, granulating surface on the inner side of the calf of the leg. Over the head of the fibula was a suppurating wound which led down to broken bone. A radiograph taken after his arrival here showed a fracture of the upper end of the tibia running into the joint with irregular callus formation and thickening of the bone. Twenty days after coming to Reading the knee was straightened under an anaesthetic and the limb put up on a Thomas' splint. No grating of bone was felt. This was followed by a good deal of pain and some effusion into the joint. Thirty-eight days after his arrival here it was noted that there was considerable thickening all round the knee joint/

joint. The wounds were gradually healing, but there still existed a certain amount of purulent discharge. The swelling had become considerably less. Eight weeks from the time of his arrival here he was again anaesthetised and the limb forcibly moved to get rid of adhesions.

Six weeks later the limb was again forcibly moved under an anaesthetic. No more than 30° of flexion were obtainable. The limb was now put upon an inclined splint. A month later the knee became very swollen and painful. Two days later he had a temperature of 103° , and there was considerable discharge from the wounds. During the next two days the temperature failed to come down. The knee joint remained swollen and tender, and it was resolved to operate. The old wounds were thoroughly scraped, free incisions were made into the tissues round the joint, and a considerable amount of pus evacuated. Drainage tubes were put in and Eusol dressings applied. Next day the temperature had come down somewhat, and all the wounds were discharging freely. The wounds were being irrigated with Eusol every two hours. There was a free discharge of pus from the wounds and the peri-articular tissues appeared to be less swollen and tender. The temperature, however, continued to rise during the next few days, and the patient became/

became very ill. The joint was now freely opened by incisions on either side of the patella and through the popliteal space. Sulphur and glycerine plugs were inserted. The temperature, however, still remained high, and, as the patient was getting steadily worse, the surgeon in charge, who contemplated amputating the limb, consented to allow the joint to be sown with the Reading bacillus.

The patient was again anaesthetised, and the two lateral incisions thrown into one by a transverse one. There was a considerable amount of dirty sloughing material in the soft tissues and the articular ends of the bone were eroded. Streptococci were obtained from the joint, but no anaerobe. The joint surfaces were washed over with saline and partially dried, after which living cultures of the Reading bacillus were liberally sown all over the interior of the joint and on the wound surfaces. Sphagnum moss contained in improvised bags of sterile gauze was packed into the joint and the edges of the wound partially brought together with one or two sutures.

Within four days the patient was feeling better. He had lost his strained, anxious look. His tongue was moist and clean and his temperature was lower.

The/

The packing was left in situ for nine days, during which time the characteristic odour was very perceptible. On removal of the packing the surfaces presented the usual appearance of wounds treated in this way. His temperature now became normal. The few thin sloughs which had not quite become free gradually disappeared and the wounds speedily filled up with firm, healthy bright red granulations.

Comments. This case is an example of a very severe injury to the parts in the neighbourhood of the knee joint without actually at the time involving that joint. There was a comminuted fracture. The wounds were dirty and a gas infection had ensued. Notwithstanding removal of a foreign body, free incisions and thorough drainage, the wounds, although they improved very considerably, failed to heal up completely. Even after five months' treatment, the wounds were still discharging. The joint itself had not hitherto become infected. During these five months various attempts appear to have been made to overcome the deformity and stiffness of the joint which had been allowed to supervene. Nothing untoward happened till after the third occasion of forcible manipulation, when the joint became swollen and/

and tender. It is to be borne in mind that the tissues, despite prolonged treatment, were still infected, and it is presumed that the rough handling of the joint lighted up a fresh infection. Apparently it was thought at first that the recrudescence was confined to the extra-articular tissues. Free incisions, scraping and two-hourly irrigation with Eusol failed to relieve the condition, obviously because the synovial cavity itself was involved. Valuable time was thus lost. When it was recognised that more radical measures were necessary, the patient was very acutely ill and stood in jeopardy of his life. The joint was then freely opened and drained. Despite this, the patient remained acutely ill, and the necessity for amputation to save the man's life presented itself to the surgeon. An alternative was suggested, viz. to sow the wound with the Reading bacillus. This was done, without any further operative interference beyond joining up the two lateral incisions which had already been made into the joint. This was done in order that more accurate packing of the joint could be carried out and to ensure that the living culture reached every part of the cavity. No bone was removed, no scraping done, and there was no foreign body/

body to be extracted. There already existed free drainage so that if the bacillary treatment succeeded, its success could not be ascribed to more perfect operative measures or to the remedying of some previous error of omission. The Reading bacillus was not found in the wound previous to sowing. In other words, the only new factors introduced were the Reading bacillus and the sphagnum moss. After sowing, the latter was packed fairly tightly into the joint cavity so as to cover all raw surfaces and the wound partially brought together with sutures. The patient began to improve from the fourth day after sowing and continued to do so, although the packing was left in situ for nine days. After removal of the packs the wound rapidly filled up with healthy granulations.

Surgical measures failed to arrest the infection, which was promptly checked by the introduction of the Reading bacillus and moss packs. The material used for packing does not apparently matter a great deal so long as it renders the wound more or less anaerobic and yet keeps it well open. I have already referred to instances where packing or packing after vigorous surgical measures had been taken, was nevertheless/

nevertheless not followed by improvement. In these cases the typical odour was absent, as was also the specific anaerobe. From a consideration of all the facts, it is, in my opinion, legitimate to claim that in the above case the sole factor responsible for effecting the rapid cure, was the actively growing Reading bacillus.

Case 14. C.W.: was admitted to the War Hospital, Reading, after having received a gun-shot wound of the right arm four days previously. There was an entrance wound situated on the outer side of the arm about three inches above the elbow, and another in the forearm about two inches below the elbow. The latter wound was the result of operation for the removal of a foreign body. There was complete musculo-spiral paralysis. The wounds cleaned up under antiseptic treatment, and in twelve days from the date of admission there were left two sinuses, one large and deep running to the elbow joint, the other $2\frac{1}{2}$ " deep in the upper arm. Nineteen days after admission, heat, tenderness and swelling appeared round the elbow joint. Both sinuses were discharging pus freely. Eusol treatment was kept up for six days longer, but as the patient's temperature had been rising for some days/

days, he was anaesthetised, and the wounds enlarged. They were then simply swabbed out with plain dry gauze after which I sowed the wound surfaces with a living culture of the Reading bacillus. They were then packed with sphagnum moss in bags moistened with normal saline. There was the usual increase in temperature generally met with after inserting packs. On the third day, however the temperature had fallen to normal. The sphagnum moss bags were removed on the ninth day. The walls of the wound showed somewhat coarse granulations, but were otherwise healthy, and gradually healed up without further incident under a daily dressing of gauze wrung out of normal saline.

Comments. In this case the wounds had cleaned up partially under antiseptic treatment. They did not do so completely, and the healing appeared to have come to a standstill. They continued to discharge pus freely, and nineteen days after receipt of the wounds there was evidence that the infection had not only not been overcome, but that there was a recrudescence of organismal activity. The antiseptic treatment was given a further chance for the next few days, but was then stopped, after having been tried for twenty-nine days, and without any other interference/

interference beyond enlarging the wounds which had contracted somewhat since admission, the Reading bacillus was introduced, followed by sphagnum moss packs. In three days the patient's temperature had come down and the wounds healed up without further trouble. No surgical measures were undertaken at the time of sowing beyond enlarging the wound orifices so as to admit the moss packs.

On the one hand we find that under antiseptic treatment employed over a period of twenty-nine days the wounds improved at first, then began to "mark time" and finally to show signs of fresh septic activity. On the other hand, within three days after the introduction of the Reading bacillus, the temperature had fallen, and on the ninth day from the inception of this treatment the wounds were healthy and granulating and healed without further trouble under simple sterile saline dressings. In this case the recovery could not be attributed to the removal of foreign bodies or of bone fragments, and the credit may fairly be claimed by the specific anaerobe introduced.

Case 15. Pte. J. S-e.: was admitted to the Royal Berks Hospital, Reading, under the care of Major Price, R.A.M.C., thirteen weeks after receiving a gun-shot wound of the left popliteal space.

On admission there was a large sloughing wound in the left popliteal space. Sensation and movement of the foot and leg were good. Three days later the radiographer reported that a shrapnel bullet was embedded in the femur slightly to the inner side of the middle line on its posterior aspect. The report gave details of its exact position for the guidance of the surgeon.

Next day, i.e. four days after admission, the foreign body was successfully removed. On the day following the operation there was considerable pain in the joint and a slight effusion into it, mainly noticeable on its inner side. The wound continued to discharge and hot boracic fomentations were applied.

A week later, owing to pocketing of pus, the wound was adequately enlarged and drainage tubes inserted. Fluid aspirated from the joint cavity on this occasion proved sterile. Fomentations were continued and the limb was immobilised in a plaster of Paris splint provided with the necessary openings for gaining/

gaining free access to the wound.

Since admission he had had almost a daily rise of temperature to 101° or 102° F. and for sixteen days before the knee joint was opened it had never been once normal. As there was no improvement, the knee joint was freely opened into on either side of the patella and free and efficient drainage secured. This operation took place four weeks after the date of his admission. Drainage tubes were inserted, and the limb was replaced in plaster. Twenty-five days later pus was found tracking along the inner side of the thigh and suitable incisions were made. Several injections of anti-streptococcal serum were given and later a sensitised autogenous streptococcal vaccine was prepared and injected. The patient continued however to run an irregular swinging temperature for the next five weeks when further incisions were made. Iodoform dressings and other antiseptics were then tried for the next six weeks. The patient, however, continued to get worse and the long-continued fever and suppuration had rendered him very weak. He had gone steadily downhill, and as a last effort to save the limb I was asked to sow the wounds with the Reading bacillus. The patient was accordingly anaesthetised and the various wounds where they had partially closed up, were/

were enlarged. They were then washed with sterile normal saline and profusely sown with living cultures of the Reading bacillus. They were then well packed with sphagnum moss enclosed in improvised bags made of sterile white gauze. The characteristic odour developed in the course of a few days. The temperature fell nearly to normal, and on removal of the packing on the sixth day became quite normal and remained so subsequently. When the packing was taken out, the moss was found saturated with thick yellow pus, but after irrigation the wound surfaces were found covered with healthy red granulations.

The oedema of the leg and thigh had sensibly diminished and gradually disappeared. The wounds closed up by granulation without further incident and were firmly healed about six weeks later. The knee-joint of course was stiff. The temperature remained normal and from the third or fourth day after sowing, the patient's general condition began to improve.

Comments. This case was one of grave injury. The joint was involved by a streptococcal infection. The foreign body present had been extracted. The joint was subsequently freely laid open. Many incisions had been made from time to time during a period of four and a half months. Immobilisation by/

by splints, and antiseptics of various kinds, had been tried. Anti-streptococcal serum had been injected by the surgeon and sensitised vaccines administered. In spite of all these measures, the wounds continued to discharge, the leg and thigh remained very much swollen and oedematous while the patient continued to run a daily swinging temperature. His general condition gradually became worse. He was critically ill and there seemed only one course open to the surgeon, viz: amputation in order to save the man's life. The patient had already undergone six operations before it was resolved to sow the wound with the Reading bacillus. This was done after simply enlarging the partially closed incisions which had previously been made from time to time. Nothing else was done beyond irrigating the wounds with sterile normal saline before sowing with the living culture. Sphagnum moss was used for packing. Within four days from the date of sowing, the patient had begun to improve. On the sixth day his temperature came down and remained thereafter normal, and in about six weeks after removal of the packing all the wounds were firmly healed. In this case the tissues had frequently been incised. Multiple openings had been made and free drainage secured/

secured, but all to no purpose. When the Reading bacillus was sown in the wounds no new operative measures were undertaken. The partly closed wounds only were re-opened, so that if the patient got better, the recovery could not be attributed either to removal of foreign bodies, pieces of loose bone, or to more efficient drainage. The only new factors introduced were the living cultures of the Reading bacillus together with a suitable inert packing of sphagnum moss. The case is typical of many. Over and over again do we see cases that have been thoroughly treated by free incisions and removal of loose bodies keep on suppurating in spite of antiseptic treatment. In the course of such a case, the need arises from time to time to make fresh incisions either to allow of the escape of accumulated pus or for the removal of further necrosed bone. The essential feature of such a case is the progressive nature of the infection. At times it seems to be recovering and then comes a fresh flare up. The end result of such a case may be recovery after a long tedious convalescence, but not infrequently in the long run the patient has to lose his limb if indeed he does not lose his life. The point I wish to/

to make clear is that the wound does not necessarily get well after thorough opening up and exposure, after evacuation of pus or removal of foreign bodies. If this were all the successful treatment of war wounds would be a simpler matter. The continued and progressive sepsis depends on something more, viz. on the pathogenic flora and the devitalised or dead soft structures.

Whatever will remove these two malign influences without further injury to the soft parts, will transform the wound from a septic condition to one where healing can take place unimpeded. This, I maintain, is exactly what the Reading bacillus achieves, and it does so in a period that can be reckoned in days instead of weeks. Nay, it does more, for by its agency the absorption of toxic substances is so diminished that improvement in the patient's general condition can be reckoned even in terms of hours.

Case 16. Pte. A. Mc.: was admitted under the care of Dr. Franklin Cox to the War Hospital, Reading, twenty-five days after receiving a severe gun-shot wound of the left leg.

The notes which arrived with him stated that the posterior/

posterior tibial artery had been injured and that the leg was full of blood clot. The leg had to be amputated on the same day as the injury was received.

On arrival in Reading twenty-five days later, the stump was found to be very dirty and in a very septic condition. The tissues were sloughing and some of the ligatures were still in situ. The amputation had apparently been performed about four inches below the left knee joint. The subsequent treatment apparently consisted of Eusol dressings. As the patient's general and local condition was bad, it was decided to sow the sloughing surfaces with the Reading bacillus. This was done and the wound packed and covered with sphagnum moss contained in gauze bags. The dressings were left untouched for six days during which time it was evident from the smell that the organism was flourishing. In two days the patient began to mend, and when the bags were removed and the stump irrigated, it was found to be covered with bright red, healthy granulations. There were one or two thin digested sloughs which came away in a day or two and healing took place rapidly without further incident.

Comments. This case was not in such a critical condition/

condition as most of the others recorded, although his general condition was bad. The leg had been amputated nearly a month before but the flaps had broken down under sepsis, and the exposed tissues were black and sloughing. It was a case that under antiseptic applications was likely to take a considerable time to get well, if indeed the septic process did not extend to and involve parts higher up, as it was even now showing a tendency to do. He was apparently suffering from the effects of the absorption of toxic substances. The longer this continued, the more were his powers of resistance likely to be lowered.

The wound was then sown with the Reading bacillus and packed. As soon as the organism had gained a footing and begun to proliferate, the patient began to improve generally and on removal of the bags, the wounds were found to be clean and healthy, although one or two emaciated sloughs were still attached by one end to the tissues. These came away in a day or two and the wound promptly healed. From the fact that a few thin sloughs may frequently still be found in these cases attached to the wound by a slender tag, it is scarcely likely that the bulk of dead tissue could be proteolysed within two or/

or three days after the introduction of the organism. It is unlikely, therefore, that the rapid improvement in the general condition of these patients should be due solely to the complete proteolysis of dead tissues. While this is part of the explanation, I feel that there is yet another. The improvement not infrequently begins in twenty-four to forty-eight hours, too soon for complete proteolysis to have occurred. In my opinion this improvement is related in some way to a reduction in the amount of toxic substances absorbed into the patient's system. Just how this reduction is brought about cannot be clearly outlined at present, but that the Reading bacillus, and that organism alone of many that I have investigated, possesses this extraordinary power, is proved conclusively, I think, by the experimental work shown in detail further on in the course of this thesis.

V. CASES illustrating successful treatment solely by introduction of the Reading bacillus after a preliminary attempt with salt-bags alone had been unsuccessful and where on the first occasion the bacillus was known to be absent.

Case 17. Pte. D. L.: was admitted to the War Hospital, Reading, under the care of Captain Foster, R.A.M.C., eleven weeks after having sustained gunshot wounds. He had been at intermediate hospitals prior to his admission to the Reading War Hospital and the following is a brief resume of the available notes. He had been wounded in several places by shell fragments, viz: in the left shoulder, epigastrium, both hands, and in the left leg in two places. A first dressing was applied six hours after receipt of the injuries. The radiographer's report showed that there was a comminuted fracture situated about the middle third of the fibula. Fragments of loose bone and metal were present. There was no bone injury in the neighbourhood of the shoulder. Later, on the day of the injury, the wounds of/

of the leg were excised, foreign bodies removed, free drainage established by a counter incision, and the wound surfaces smeared with B.I.P.P.

The wound in the shoulder and that in the epigastrium were excised and a foreign body extracted from each. It was also found necessary to amputate two fingers, one from each hand. During the next few days the wounds, with the exception of the upper of the two leg wounds, appeared to be fairly healthy. The patient developed corneal and conjunctival ulcers as a result of injury to the eyes by sand grains. The leg was put up on a Thomas' splint and nine days after being wounded he was admitted to the Cambridge Hospital. On admission to that hospital, the wounds in the shoulder and epigastrium were superficial and clean, and the amputation stumps were nearly healed. The lower wound of the leg was beginning to look clean, but the upper wound was dirty and there was bare bone at the bottom of it. For some days thereafter the patient ran a temperature and he complained of pain in the leg. The leg wounds then gradually began to heal and after a six weeks' stay in the Cambridge hospital he was sent to Bearwood, Berks, able to get about on crutches. At this stage, all his wounds were healed, except those in/

in the leg, and they were healing. The patient's general condition, however, was regarded only as "fair." The treatment at Bear Wood consisted in the application of wet dressings, but whether saline or Eusol was employed has not been stated. After a four weeks' stay at Bear Wood the leg began to cause the patient considerable pain, and he commenced to run a temperature at times above 103° F. He was at this point admitted to the War Hospital, Reading, with what was then regarded as a subacute osteomyelitis of the left tibia. An autogenous vaccine was prepared from streptococci recovered from the wounds, and this was followed by an apparent improvement. As the man was obviously ill, and his temperature did not remain down, an operation was undertaken eleven weeks from the date of receipt of the injury. The whole of the tibia was found to be involved, the upper third being a mere shell of bone. The remainder of the bone was freely opened along its entire length. A large abscess in the calf of the leg communicating with the medullary cavity of the tibia was evacuated. A counter opening was made posteriorly the whole wound well irrigated and packed with salt bags. As a result of this the temperature fell a little. The salt bags were left in situ for five days/

days and vaccine was still persevered with, but although there was some improvement, the leg still caused pain, the man's pulse was poor and the temperature still remained up. He was not making satisfactory progress. The characteristic odour associated with successful cases was absent. It was then decided to sow the wound with a living culture of the specific anaerobe.

On removing the salt packs, the dressings were seen to contain a good deal of blood and while irrigating the wound with saline, secondary haemorrhage occurred. The vessel was secured, but there was some difficulty in doing so as the tissues were so friable and rotten. The wound which extended the whole length of the leg, was covered with large dirty sloughs and looked altogether in a very unhealthy state, the secondary haemorrhage testifying to the condition of the wound. Without doing anything further beyond taking a sample of the pus, the whole surface was very liberally sown by me with an old culture of the specific anaerobe and the wound again packed with fresh salt bags in the way recommended.

The temperature for the next two or three days rose slightly higher than before, and then came down to fluctuate between 99° and normal. The patient began/

began to feel better and looked better. The characteristic odour became recognisable about the fourth day, but the packing was not interfered with for eight days. The pus which had been taken after removal of the first set of salt bags was examined, and from it I recovered staphylococcus aureus and streptococcus longus. I was unable to grow any anaerobe from it.

When on the eighth day I removed the second set of salt packs, they were found as usual to be bathed in pus from which staphylococci, streptococci and the specific anaerobe were recovered. The wound was then irrigated with Eusol and it was then seen that the surfaces were lined with bright red granulations covered here and there by thin membranous sloughs which came away easily within the next day or two, so that the whole wound had a bright healthy red appearance and granulations rapidly began to fill up the large cavity. The knee joint had not become involved, and the patient's general condition was excellent.

Comments. This case has been included mainly to emphasise the fact that recovery followed the implantation of the Reading bacillus when the conditions/

conditions had been rendered favourable for its growth. The improvement which began after sowing with the anaerobe could not be attributed to operative interference, for all the operative interference that was necessary had on a previous date been thoroughly carried out. Those operative measures were aided by the packing of the large wound with salt bags. Improvement, however, did not follow, as is evident from the state of the wound on removal of the salt bags. Secondary haemorrhage indeed actually occurred. Then without further interference in any other way, and this is the point, the Reading bacillus was introduced and fresh salt bags inserted. The wound now began to smell and improvement followed. On removal of the bags the wound surfaces were found covered with healthy granulations, and within a few days presented the appearance of clean raw beef.

There can be little doubt that the change was effected by the agency of the Reading bacillus.

The case also illustrates another point which has been noticed more than once, and to which reference should be made, viz: that cases treated over a long period with antiseptics apparently almost reach/

reach the point of recovery when a fresh recrudescence of the septic process occurs. This may happen not once but several times in the course of the wound's history and not infrequently necessitates several minor operations at various times. This adds very considerably to the period of convalescence.

With the Reading bacillus on the other hand, where a proper environment by means of packing has been secured, the wound quickly becomes clean and covered with healthy granulations. Further there is no recrudescence if, at the time of sowing, the bacillus has been implanted in every part of the wound. Collections of pus must be opened into, otherwise the bacillus can never reach them.

Case 18. Pte. F—y: was admitted to the War Hospital, Reading, seven days after receiving a severe gun-shot wound of the right leg. On his arrival in Reading there was a huge gaping wound with everted oedematous edges showing many fragments of broken bone at its base. The wound was very dirty and was discharging brown-coloured, bubbling, evil-smelling pus. The wound was situated at the junction of the upper and middle thirds of the right leg on its anterior surface. Posteriorly there was a smaller circular wound/

wound communicating with the former and situated rather lower down the leg. Both tibia and fibula were severely comminuted and a considerable piece of the tibia was missing. The wounds showed a tendency to bleed.

Amputation was performed through the seat of fracture on the day of the man's arrival in Reading. The tibia and fibula were seen to be hopelessly comminuted for a length of five inches. The muscles and other soft tissues were disorganised and crepitant. The flaps were accordingly left open.

The patient's general condition began thereafter to improve and the stump became cleaner. It was being irrigated with peroxide of hydrogen and dressed with gauze soaked in normal saline. Five weeks after the operation the wound looked cleaner, and pain had disappeared. It was then apparent that the bones would have to be shortened before they could be covered in. Seventeen days later re-amputation of the bone was performed. Seven weeks after the first amputation the tissues composing the stump became swollen again. Olive oil and iodoform dressings were applied by the surgeon in charge. Later this was changed to hypertonic saline dressings which/

which in turn were replaced by boracic fomentations. The wound, however, continued to discharge. The whole surface was dirty and a considerable part of it in a sloughing condition. The stump was still much swollen. It was now seven months since the first operation of amputation had been performed. Salt bags were now applied and were left in situ for five days. The characteristic odour did not develop and when the bags were removed, the wound presented much the same appearance as before they were inserted. There were no red granulations, and if anything the area of sloughing had extended. Boracic fomentations were now applied and continued for eight days. The wound became somewhat cleaner, but there was still a considerable amount of thin yellowish purulent discharge. The wound surfaces were dark coloured and dirty and there was no appearance of healing. Cultures made at this point failed to demonstrate the presence of the Reading bacillus. The wound surfaces were now sown with a living culture of the Reading bacillus and immediately packed with salt bags. Nothing further was done. These were left in situ for seven days, during which time the characteristic odour was very much in evidence/

evidence, proving the vital activity of the anaerobe. At the end of this time the salt bags were removed. The stump was now seen to be less swollen, and after irrigating the wound surfaces, healthy granulations were seen together with a few thin, emaciated yellow sloughs. Normal saline dressings were now substituted, and eleven days later skin grafts were planted. Most of these took and the wound became satisfactorily healed.

Comments. This case furnishes valuable evidence of the remarkable changes brought about by the vital activity of the Reading bacillus. It was an exceedingly severe wound at the outset. From the surgical point of view the leg was hopelessly shattered, and in addition to the usual sepsis there was a gas infection.

Free incisions were made into the tissues, and amputation performed. The patient's life was saved. Seven weeks later a further amputation was found necessary. The stump, however, remained swollen and the whole wound was thoroughly septic and in a sloughing condition. Various methods of treatment were carried out over a period of seven months - ample opportunity for testing the value of these methods. In spite of them the wound refused to heal. The/

The stump was still swollen, and the wound surfaces generally were in a septic sloughing condition. Without further treatment salt bags were applied. These, however, were equally ineffective. The typical smell associated with the living presence of the Reading bacillus did not develop. On removal of the bags it was evident that the condition of the wound was much the same as it was before, furnishing further proof that the specific bacillus had not been at work. The stump was still swollen. Cultures made failed to show the presence of the specific anaerobe.

To recapitulate, radical surgical measures had been carried out on at least two occasions. Various methods of treatment had been given a chance for seven months. Salt bags applied to the wound in the way described failed to effect a change. The Reading bacillus did not appear to be present, judging from the absence of the characteristic odour and from the unchanged aspect of the wound, and it was not found in cultures made at this stage. Without further surgical interference the wound was simply sown with a living culture of the bacillus in question, and packed as before with salt bags. The/

The clinical course of events then underwent a complete change. The typical odour developed showing that the organism was at work. Further proof was forthcoming in the shape of a gradual diminution in the swelling of the stump. In eleven days after removal of the salt packs the wound was clean, healthy and in a condition to be skin grafted after which it healed up without further incident. In this case no surgical interference was considered necessary on either occasion of using salt bags. The only new factor introduced on the second occasion of packing was the Reading bacillus in living culture, so that again no adverse critic might have occasion to cast doubt on the means which effected the improvement.

The Reading bacillus had accomplished in a few days what other methods had failed to do in the course of months.

Case 19. Pte. G. T.: was admitted to the War Hospital, Reading nine days after receiving a gunshot wound of the left arm.

On admission his condition was as follows:

The/

The left upper arm was very much swollen. There was a small entrance wound situated about three inches above the external condyle. There was a huge wound of exit situated over the posterior surface of the elbow. Both wounds were very septic and dirty-looking and there was a discharge of thin brownish coloured pus. There was a compound fracture of the humerus.

The pulse was rapid and the temperature high. Later, on the same day, the patient was anaesthetised, the wounds were freely opened up and several loose fragments of bone were removed. The cavity was then packed with salt bags. The bags were left in situ for eight days and although there was a slight improvement in the patient's condition, the typical odour did not develop, his temperature remained up and the arm was still swollen.

Without doing anything beyond irrigating the wound with sterile normal saline and drying partially with plain sterile gauze the wound was freely sown with a living culture of the Reading bacillus and packed with fresh salt bags. The typical smell characteristic of the actively growing Reading bacilli/

bacilli developed in three days. The temperature and pulse became normal and the patient's general condition was greatly improved. The temperature however went up again and oedema of the fore-arm persisted. The salt bags were left undisturbed for five days, at the end of which time they were removed. After irrigating with Eusol, the wound surfaces looked perfectly clean and were seen to be lined with healthy red granulations. The exposed bony fragments were white and clean.

Nine days later, as the temperature, although lower than it had been, was still raised and the forearm appeared swollen, an incision was made over the radius when pus was found. Without resowing, this fresh wound was packed with salt bags. The characteristic odour developed. The temperature came down in three days and stayed down, and the swelling disappeared. In six days the bags were removed and the wounds healed up without further trouble.

Comments. This case again is instructive and furnishes points of interest. To begin with, the wounds were very septic and there was comminution of the lower end of the humerus. Removal of broken fragments and packing with salt did not bring down the temperature or influence the condition of the wounds/

wounds. They were then sown with the Reading bacillus without further complicating interference. This was followed in three days by a fall in the temperature to normal and by the usual improvement in the patient's general condition. But the improvement was not maintained. Nevertheless when the bags were removed the wound was perfectly clean and covered over with healthy granulations and the exposed bone was white and clean. The condition of this wound was obviously not the cause of the temperature, and the presence of oedema of the forearm suggested that there was sepsis in this region, which, because of its slight character had been overlooked at first, especially as attention was focussed mainly on the obvious wound. On opening over the radius some pus escaped. This wound was now packed. The Reading bacillus became active and this wound followed the course common to all successful salt bag wounds. The case illustrates a point on which I have laid some emphasis viz. that the Reading bacillus will not penetrate a barrier of living tissue and so influence a septic focus shut off from the wound in which it is first implanted. Such a focus/

focus must be exposed and the bacillus then introduced. As soon as it was recognised in this case that a septic focus had been overlooked, steps were taken to bring the bacillus into direct contact with it. It was considered that as the organism had already been growing profusely in the adjoining wound, and the spores were probably freely distributed in the neighbourhood, nothing more would be necessary beyond packing the fresh wound. This expectation proved correct, for the bacillus at once commenced to grow.

Having brought the organism into direct contact with the infected surfaces, the damaged tissues were speedily acted upon by it, the absorption of toxins came to an end, the progress of the pathogenic infection was checked and the wounds became healthy and began to heal up.

Case 20. Pte. A.: was admitted to the Reading War Hospital, seventeen days after receipt of gun-shot wounds of the left leg and chest. There were two clean wounds situated above the left ankle, together with a fracture of the fibula. There were several other wounds situated on the posterior aspect of the leg and thigh, three clean wounds of the left side of the/

the chest and a small punctured wound on the anterior aspect of the right thigh from which pus was being discharged. Nine days later it was noticed that the wound above the left ankle had begun to suppurate, and two days thereafter, as the patient's temperature continued to show a daily rise, operative interference was considered necessary. An abscess was evacuated from the region of the left ankle, and a foreign body removed. Having been thus freely laid open, the wound was packed with salt bags. These were left in situ for six days without interference. Meanwhile, however, no characteristic odour developed, and the temperature showed even greater daily fluctuations than before. The salt bags were accordingly removed, and it was now seen that there were considerable swelling and oedema of the foot and ankle. From cultures made from the pus at this stage, I was unable to isolate the specific anaerobe. On the day following removal of the salt-packs, the wound was re-opened, irrigated and the cavity dried out with sterile gauze. I then sowed the wound liberally with a living culture of the specific anaerobe and, at my suggestion, instead of salt, sphagnum moss packs moistened with sterile water were inserted by the surgeon. The packs were left in situ for six days, and the characteristic odour, previously absent during treatment with the salt/

salt packs, became very perceptible. The temperature began to fall on the third day, and the patient was quite comfortable except for slight pain in the wound commented on below.

When the dressings came to be removed, the superficial layers of gauze were found to be set quite hard and board-like, exactly as in the case of wounds treated by the salt bag method. The packing was bathed in yellow pus. The wound surfaces were covered with a greyish yellow purulent discharge, but, on washing this away with Eusol, they were found to be covered with healthy red granulations. All swelling of the foot and ankle had disappeared. All the other wounds, with the exception of one in the leg, were in a healthy healing condition. The exception, although not of such a serious nature as the one just discussed, was used as a control for the ankle wound. The control was not treated with the specific anaerobe, and it is interesting to note that it remained in an unhealthy suppurating condition for a considerable time after the ankle wound was healed. It eventually got well.

Comments. This case is an important one inasmuch as it illustrates the point that I have been trying to emphasise, viz: that free opening of a wound, evacuation of pus, removal of foreign bodies and subsequent/

subsequent plugging with salt bags, are not necessarily followed by improvement. The latter apparently does not depend either on thorough surgical measures short of excision or on the presence of salt with all its reputed beneficial action. There appears to be some other factor, which in my opinion is the factor of vital importance. In connection with the want of success under the above treatment, is to be noted the absence of the peculiar odour characteristic of salt-bag treated wounds which are doing well. In conjunction with this must be taken the fact that the specific anaerobe was absent in culture.

In this case antiseptic treatment had been carried out over a period of 28 days without resulting benefit.

The infection was progressive to judge from the increased suppuration and from the persistently fluctuating temperature.

Having given antiseptics their opportunity so long as the surgeon deemed it safe, he decided to take more radical measures.

Accumulations of pus were evacuated, a foreign body removed and the wound very freely opened. This in itself might be expected to bring about a fall/

fall in the patient's temperature and to lead to recovery if we accept the view of those who are inclined to attribute the rapid recovery in successful salt-packed wounds to some minor surgical operation at the time of packing. This wound, however, after being thoroughly treated from a surgical point of view was then packed in the normal way with salt bags. If the case had then begun to improve, adverse critics might have attributed the recovery to the surgical interference, But it did not improve.

For six days the bags were left in situ, Instead of improvement the temperature began to show even greater fluctuations than before. Oedema and swelling increased. On removal of the packs the wound was still in an acutely septic condition.

What is of the utmost significance, the wound did not emit the characteristic smell associated with the activity of the Reading bacillus and that specific anaerobe was absent from cultures made at this point.

Now comes the crucial test. Without making any further incisions or doing anything of a surgical character beyond irrigating the surfaces to wash away discharge, and subsequently partially drying them, the wound was liberally sown with a living culture of the Reading bacillus and then packed with an inert substance/

substance, viz: spagnum moss.

The current of events was absolutely changed. The effect was almost magical. The wound began to give forth the characteristic odour indicating vital activity on the part of the Reading bacillus, the patient's temperature began to fall on the 3rd day, and the swelling and oedema to diminish. On removal of the moss bags, the wound surfaces were lined by healthy red granulations. As the moss was an inert substance and it did not matter a great deal whether salt or moss was used as a means of bringing about anaerobic conditions, the single additional factor was therefore the Reading bacillus.

Even though this case stood alone, it is sufficiently clear cut and definite to justify the deduction that the improvement was due solely to the vital action of that organism.

The point I wish to emphasise is that thorough opening up of the wound, followed by salt or moss packing, is not necessarily attended by success. From cases so treated which do yield immediate and good results, the specific anaerobe can be recovered. Where such a method has been tried without success, it will be found that the specific anaerobe is not present, but if it be then sown and the wound again packed, improvement will certainly follow.

This/

This case also demonstrates that salt is not an essential factor, either by reason of its supposed virtues, as explained by Wright, or because of its necessity to the proliferation of the anaerobe. In fact, experiments which I have carried out and which are recorded elsewhere in this thesis, demonstrate that too great a concentration of salt inhibits the activity of the Reading bacillus, although it does not kill its spores.

That salt is unnecessary seems further indicated by the fact that a substance like sphagnum moss will do equally well, the action of both forms of packing probably depending on their power of rendering the wound sufficiently anaerobic for the specific organism to proliferate freely.

Clinically there seems to be one point of difference between cases treated with salt bags and those packed with sphagnum moss. In the former, pain and smarting occur in the wound only within the first twenty-four hours, presumably due to the high concentration of salt present during part of that time. In the latter, the pain is experienced somewhat later, and is probably attributable to the swelling of the moss consequent on imbibition of fluid. This leads to some increased tension in the wound. Further experience, however, will probably enable/

enable the operator to judge exactly how much should be inserted to ensure contact with every part of the wound without subsequently becoming so tight as to cause pain. Sphagnum moss, however, seems to possess certain advantages over salt. Owing to its structure it is capable of absorbing a very great quantity of fluid. This not only converts the moss, which really consists of innumerable empty chambers into what is a solid plug. Its scaffolding of pectin prevents it from collapsing, and thus owing to expansion due to the fluids accumulated in its millions of small chambers, it acts not only as an efficient anaerobic cork to the wound, but it also acts as a splint, stretching slightly the wound surfaces so that the organism is enabled to come into contact with every part.

It is instructive to note that in this case there was a control wound which was treated in the usual way with antiseptics, and which was the last to heal. It was still in an unhealthy condition for a considerable time after the wound treated with the anaerobe was firmly healed.

CHARACTERS and PROPERTIES of the SPECIFIC
ORGANISM referred to in the previous
pages.

To begin with, this organism, as I have already indicated, belongs to the group of spore-bearing anaerobes - the presence of which in gun-shot wounds has been one of the most outstanding features of war infections. From its apparent inability to flourish and grow in living tissues, from its constant association with the presence of necrotic material in infected wounds, one has little difficulty in placing it amongst the group of saprophytes. To this group probably belong most of the spore-bearing anaerobes found in infected gun-shot wounds. They probably all agree in one point, viz: the need for dead material on which to live, and as a corollary to this it has been observed ^{20.)} that such anaerobes tend to diminish in numbers and finally to disappear from wounds as they grow older, and from which presumably the basis of dead material has vanished.

They differ, however, in their action on dead tissue; and probably also in respect of the resulting products of that action. ^{21.)} Recognising this, Henry classifies all war wound anaerobes into two great groups - the Saccharolytic and the Proteolytic, a distinction based on the fact that in the former group of/

of anaerobes the attack is mainly on the carbohydrate quantum, whereas in the latter, proteolytic activity is predominant. This is a convenient method of grouping, but it does not furnish much help when we come to identify the various members which make up groups.

From what has been said previously, and from the description about to follow, it is apparent that the specific organism which I have isolated belongs to the second or proteolytic group.

When we endeavour to identify it more closely with one or other of the various anaerobes that have been described, we come face to face with a difficulty. It does not appear to correspond absolutely with any one of those usually described in the text-books. Probably on account of the comparative rarity with which anaerobes have hitherto been encountered, they have not, with a few outstanding exceptions, received the same amount of attention or careful study that have been devoted to the aerobic series with which we are more familiar.

Consequently, while various types have been described, often imperfectly, very little or no attempt has been made to classify or to arrange them. The literature on the subject is, to say the least of it, rather/

rather bewildering. Probably the only serious attempt to render the subject intelligible is contained in Von Hibler's book,²²⁾ and recently, using this as a basis, Miss Muriel Robertson, in an extremely valuable contribution, has suggested a classification of anaerobes into four main groups. In order to arrive at some semblance of order, Miss Robertson proposes that the various strains encountered be subjected in the first instance to a uniform series of tests. In gathering the materials for her paper, Miss Robertson²³⁾ has been struck, as everyone is who looks into the subject, with the looseness which has characterised much of the work on anaerobes, and with the contradictory statements expressed concerning the morphological, cultural and biological characters of organisms supposedly of the same type. This, as she explains, is due either to extraordinary pleomorphism on the part of the anaerobes or to the fact that the conclusions arrived at by various observers, are based on a study of cultures which were in reality impure to start with. That this latter alternative is more likely to be the correct one, is illustrated by the instance which she gives of Klein's *b. Enteritidis Sporogenes*. The latter, in Klein's hands, gave variable and anomalous results, the key to which was supplied by Von Hibler when/

when he showed that Klein's culture of *b. Enteritidis Sporogenes* really consisted of two different organisms!

That the difficulty of obtaining spore-bearing anaerobes in pure culture, is a very real one, must be apparent to all who have tried to work with them.

The failure to realise the need for extreme care in demonstrating the purity of the organism as the first step in a study of its life-history, probably explains many of the conflicting statements and results.

Recognising the need for attention to this fundamental principle, and in view of the fact that, with my colleague's consent, I intended to introduce living cultures into open wounds, I made every endeavour to assure myself of the purity of the organism. The means which I devised to separate it from the spore-bearer with which it was living in symbiotic union I have already described, and what follows in the succeeding pages is based on a study of the organism so isolated. From a study of Miss Robertson's paper, it will be seen that the organism probably belongs to what she calls Group D, the proteolytic group which embraces, *b. Oedematis Maligni* (Koch), *b. Cadaveris Sporogenes*, *b. Tetani* and *b. Botulinus*. By reason of their toxic properties, it can readily be differentiated from the two latter. With regard to its identification with one or other of the two former there/

there is greater difficulty. The fact that *b. cadaveris sporogenes* is described as a long slender rod with round terminal spores seems at once to offer a valuable point of differentiation, and one that is sufficient without any reference to its pathogenic or non-pathogenic properties. We are left therefore with one member of the group, viz., the *b. oedematis maligni* of Koch, which appears to be identical with the proteolytic strains isolated by Miss Robertson from a certain number of war wound infections. The organism which I have isolated agrees closely in almost every respect both with Miss Robertson's strains and with the description of the *b. oedematis maligni* of Koch, and while it may eventually be proved to be the same organism, it appears to differ slightly in two points, viz., in its reaction in milk, and in its effect on animals. So far, I have been unable to demonstrate that it possesses any pathogenic action. For this reason, I do not at present feel disposed to follow Miss Robertson's lead in stating that it is definitely the *b. oedematis maligni* (Koch.) In view, moreover, of the fact that there appear to be undoubted strains of a bacillus similar to Koch's, but differing in the fact that they are non-pathogenic to animals, I have determined to call my organism the Reading bacillus. This is a non-committal name, and can be easily/

easily abandoned if it be shown later that the organism is identical with one that has been described before, and which already possesses a distinctive title. I am quite aware that the Reading bacillus may have been previously described and that the honour of its discovery may belong to some one else. The main point on which I wish to lay emphasis is the fact that it can be enlisted in the services of the surgeon - a possibility to which I am not aware attention has hitherto been directed. Since starting to write this thesis, I have discovered other references in recent literature to organisms which appear to be very similar to the Reading bacillus. The first occurs in a paper by Fleming based on a study of cases of gas gangrene which he investigated at Boulogne working in Wright's research laboratory. Amongst other anaerobes isolated from such wounds are two which he designates bacillus "X" and "Y" respectively. Unfortunately, he does not give any description of them, beyond saying that they are both anaerobic and spore-bearers, but, judging from one of the illustrations in his paper, the organism called by him bacillus "X", resembles morphologically at least the Reading bacillus. Without any further differentiation, he states that they are largely responsible for the foul/

foul smell of gangrenous wounds, and that he has never been able to demonstrate any pathogenic action on their part. *B. aerogenes capsulatus*, on the other hand, was in every case the predominant anaerobe present, and was found by him to be the only one capable of reproducing the disease in animals. The second reference occurs in a paper by Prof. Dean and Captain Mouatt, R.A.M.C.T. This paper deals with the bacteriology of gangrenous wounds under treatment in Sheffield. While a few showed clinical symptoms of emphysematous gangrene, the majority were presumably similar to the wounds observed in Reading. The description which they give of the organism isolated by them, seems to correspond in all essential points with that of the Reading bacillus. Their observations on the part played by this organism in septic wounds are interesting and seem to a certain extent to support the arguments set forth in this thesis.

They say that "there is apparently no reason to think that the presence of this micro-organism in a wound is necessarily of serious import. This conclusion is substantiated by the results obtained by the inoculation of animals. Its presence is an indication of the occurrence of gangrene, for it has probably little or no capacity for multiplication in/

"in the living tissue. There is no evidence that
"its presence is constantly associated with any
"particular group of signs or symptoms."

They employed eight different strains with which they inoculated 13 guinea-pigs and 2 rabbits. All the animals, with one exception, were alive and well a month later. The animal which died showed no evidence of any lesion as a result of the inoculation. From this they conclude that the organism cannot be considered parasitic in the sense that it is capable of multiplying in living tissue. In dead tissues, however, they seem to think that anaerobes produce by their ferment action, poisonous substances, possibly organic acids, which progressively damage the living structures. It will be seen from this that they do not apparently discriminate between one anaerobe and another, nor do they take into account the fact that some may not give rise to toxic substances. This brings us back to a point which is still little understood and until it is understood, the subject of anaerobic wound infections and the best treatment therefor will continue to provide problems for elucidation. Because some are harmful, it does not follow that all are, and a recognition of this fact is essential in order to understand my point of view as set out in this thesis.

In/

In a third paper,²⁶⁾ Kenneth Goadby gives a brief description of the characters by which he identifies as *b. oedematis maligni* organisms recovered from war wounds. His description, so far as it goes, seems to suggest that it may be the same as the Reading bacillus, but as he states that it may have round or oval spores, it may be legitimately assumed that he has not succeeded in isolating a pure strain.

There are many indications that an organism or organisms apparently similar to the Reading bacillus, have been observed by other workers. While there has been a tendency to look upon it as non-pathogenic, others appear to regard it as responsible, in part at least, for the production of gas gangrene, from which it is obvious that a great deal more is necessary in the way of investigation before these two opposing theories are reconciled. The first step, I have said, is to isolate a particular anaerobe in pure culture. The next step will be the application of a certain uniform series of tests in order to identify the particular anaerobe more or less closely with one of the standard types. The third step will probably be, its differentiation into pathogenic or non-pathogenic groups or the determination of the factors which, if it is not invariably non-pathogenic, render it on occasion pathogenic. Doubtless in the course/

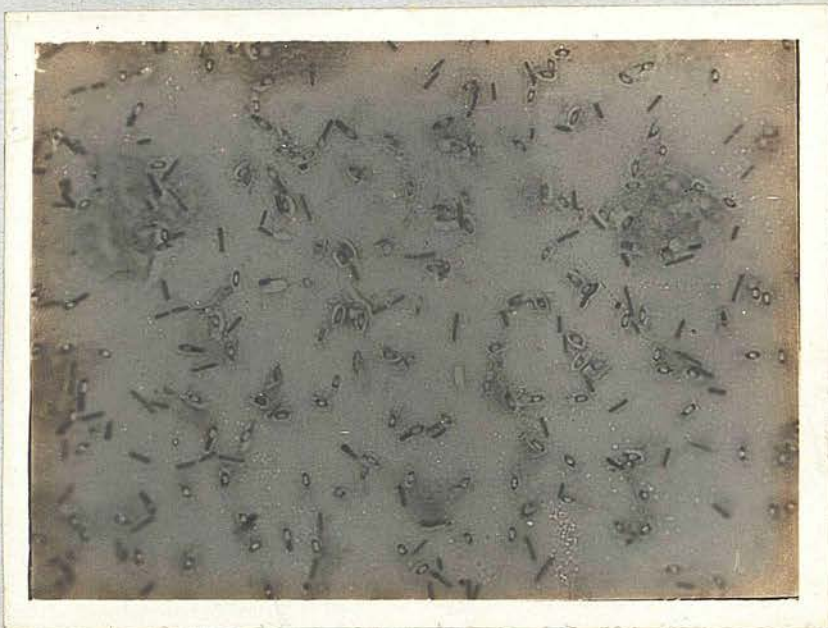
course of this work more delicate criteria may be found whereby further subdivision may be attained. The first step I have endeavoured to take, and so far as I can judge, have done so successfully. The second step, viz., the subjecting of the Reading bacillus to a uniform series of tests, is included in the following pages. As regards the third step, I have endeavoured throughout this thesis to show that it is innocuous both to animals and to man when applied under the conditions already mentioned.

Morphology:-

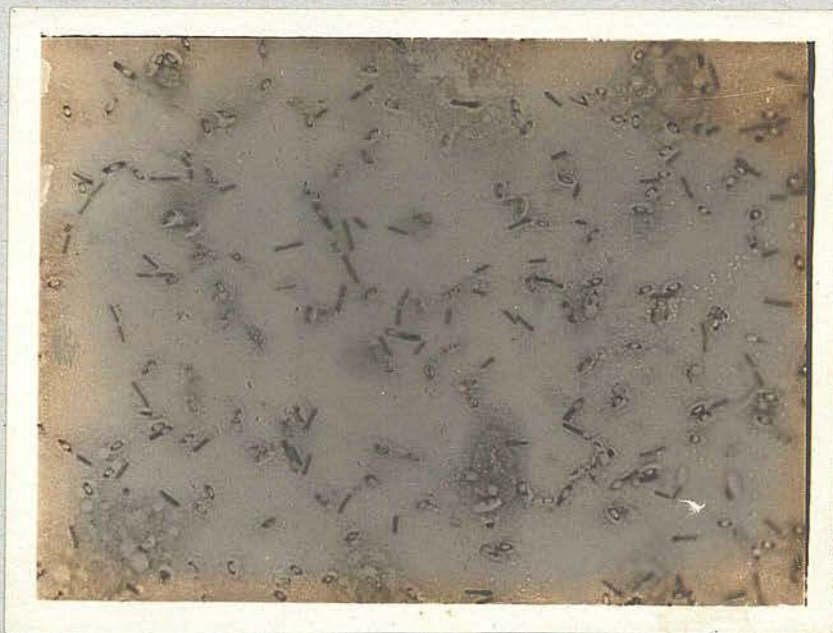
The organism consists of a rod, having a fairly large oval subterminal spore. The actual shape is probably best seen in hanging-drop preparations, when the body of the bacillus appears to be somewhat torpedo-shaped. The blunter and broader end corresponds to the situation of the contained spore, and from this the body gradually narrows to the opposite extremity. In dried stained films, on the other hand, the oval spore appears to bulge out at either side beyond the contour of the bacillary body. This, however, is probably an artefact due to shrinkage in the course of drying.

In/

5.



6.



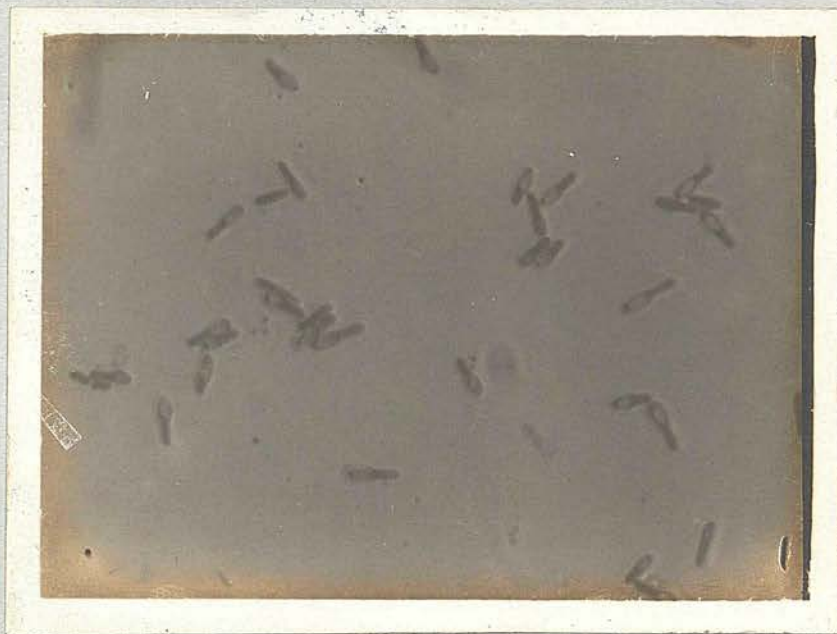
5 & 6. The Reading Bacillus at the end of 48 hours incubation in maltose broth. x 1000. Gram.

Sporing, non-sporing and clostridial forms are seen as well as free spores.

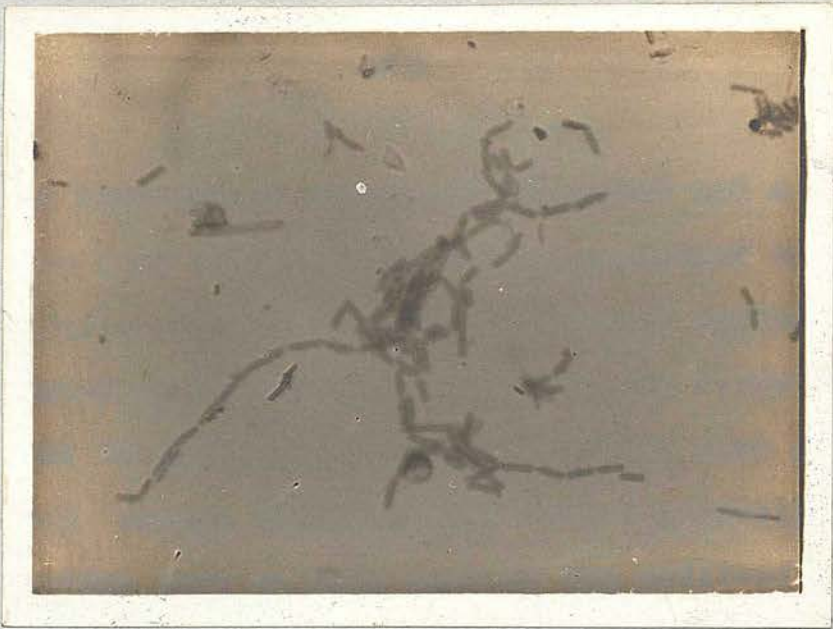
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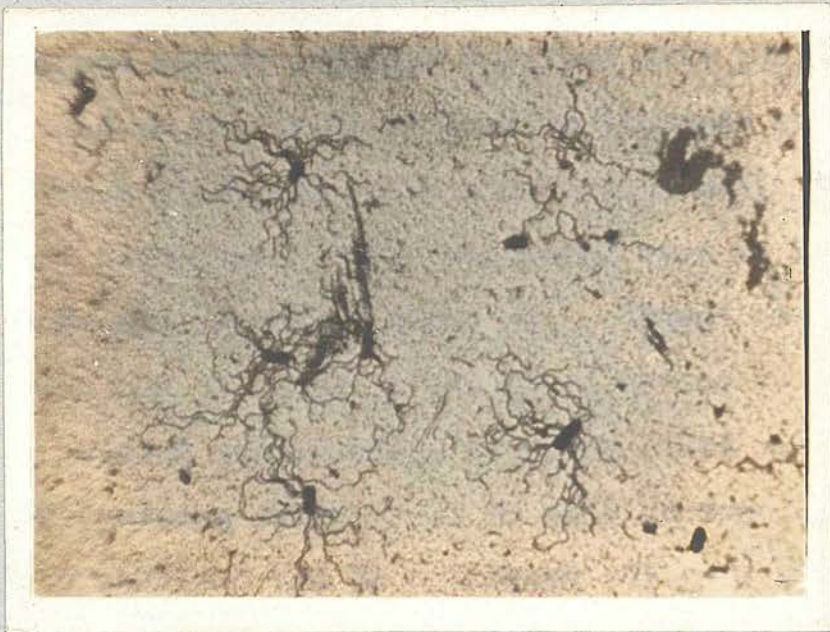
8.



7 & 8. Microphotographs of films
made from agar cultures.
X 1500. Gram.



9. Showing Chain formation.



10. Showing flagellae, from an
agar slope culture 24 hours
old. X 1500.

In a few instances the spore is situated centrally, while the bacillary body tapers away to each extremity, - the so-called Clostridial form. Occasionally one may be seen where the oval spore appears to be terminal, but as a rule there is always a small tag of bacillary body projecting beyond the contained spore. Now and again a bacillus may be seen with an oval subterminal spore at each end. The bacillus is provided with numerous long wavy lateral flagella which, when seen detached, closely resemble spirilla with fairly regular undulations. Under certain conditions there may be found along with isolated bacilli, chains composed of bacillary elements of varying size, one or two of which may also show spore-formation. The chain form may be replaced in some instances by an unsegmented filament of varying length. In young cultures, non-sporing bacilli of course predominate, while in old cultures, especially those grown on solid media, nothing remains but free spores, some of these still showing chain formation with the suspicion of the outlines of a filament in which they had apparently been formed.

This considerable variation in shape and arrangement, as well as spore-formation, probably depend on the composition and character of the medium employed, and on the length of time it has been incubated.

Size./

Size.

The average length of the sporing bacillus varies from 2.5 to 5.3 μ with an average breadth of 0.5 to 0.9 μ . Free spores measure on an average 1.46 μ long by 1.02 μ broad. These figures relate to the organism as seen in the fixed and stained condition, but of course there is considerable range of variation, both larger and smaller sizes being observed.

Motility.

The bacillus, both in its sporing and non-sporing forms, is actively motile, having a sort of "waddling" movement. This power of motility persists apparently for a considerable length of time.

Staining properties.

It stains easily with the ordinary anilin dyes and is not acid fast. In young cultures it is Gram-positive, but in older cultures tends to be less markedly so, and may even be definitely Gram negative.

CULTURAL CHARACTERS.

The organism is essentially ^{an} anaerobe, and will not grow under ordinary aerobic conditions. In meat-tubes, in order to secure the necessary anaerobic conditions, I employed liquid paraffin as a supernatant fluid covering. Such tubes were then heated to drive off any imprisoned air. As such tubes, however, were unpleasant to work with, I gave up this method for incubation in some form of anaerobic apparatus. Subsequently, I found that the Reading bacillus would grow in meat-broth tubes without any special precautions being taken to deprive the surrounding atmosphere of oxygen. This is practically Tarozzi's method ²⁷⁾ of growing strictly anaerobic organisms, although he used pieces of freshly excised organs in place of cooked meat. In the case of other media, however, it is necessary to put them up in some form of anaerobic apparatus. As the laboratory apparatus consisting of a Bulloch's apparatus, was not nearly adequate for the large numbers of culture tubes which I had under observation, I was forced to devise some other form of apparatus which would hold more tubes, and at the same time would occupy less incubator space.

AUTHOR'S APPARATUS for GROWING ORGANISMS
under ANAEROBIC CONDITIONS.

1. For culture tubes.

I made use of empty 2-lb. "Glaxo" tins, of which I had a considerable number. Such tins can be quickly transformed in the following way.

Two holes are punched out of the lid, and two right-angled metal tubes inserted and soldered in position in such a way that one of the tubes projects some distance downwards below the lid. To this is attached a piece of india-rubber tubing, which passes nearly to the bottom of the tin when the lid is on.

To the right-angled limbs projecting from the lid are fitted two pieces of rubber tubing with clips attached.

The line of junction of the bottom of the tin with the body requires a little solder run round it as does also the vertical junction on one side of the body.

The lid of a Glaxo tin fits tightly down on a ledge and when in position has a slight flange projecting over a shallow trough which runs all round the edge of the lid. It is a very simple matter to render/

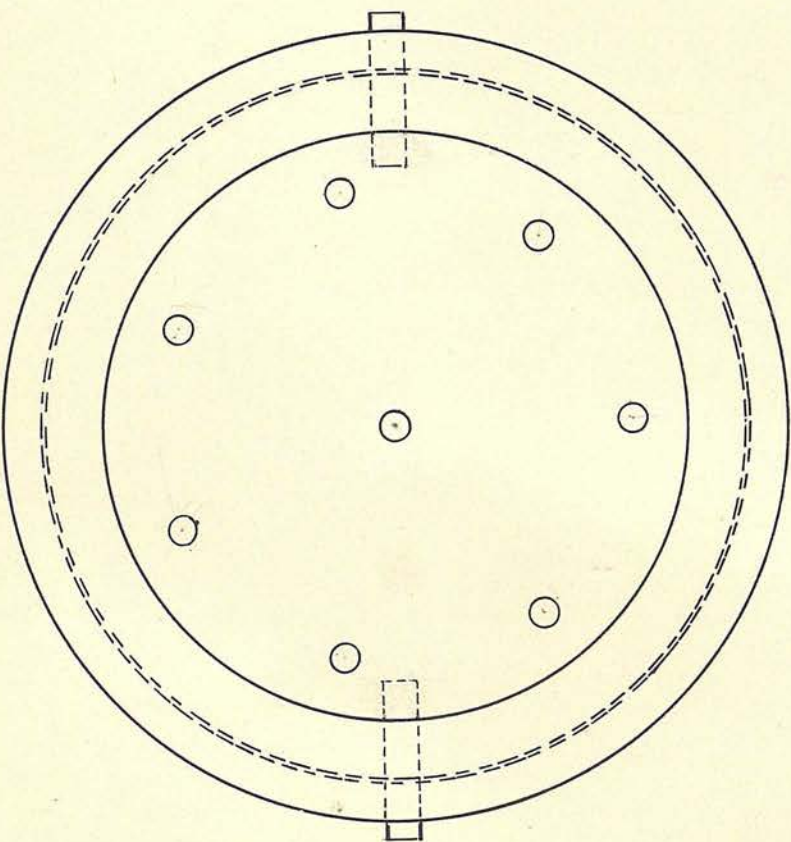
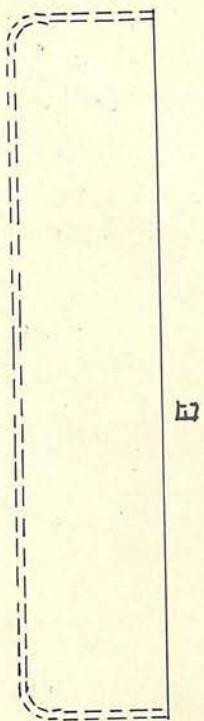
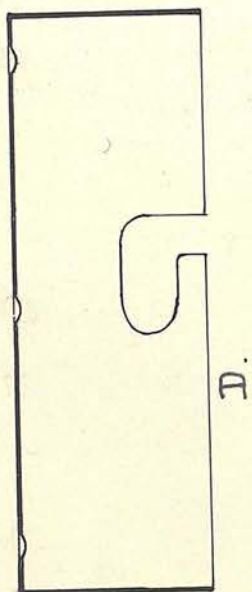
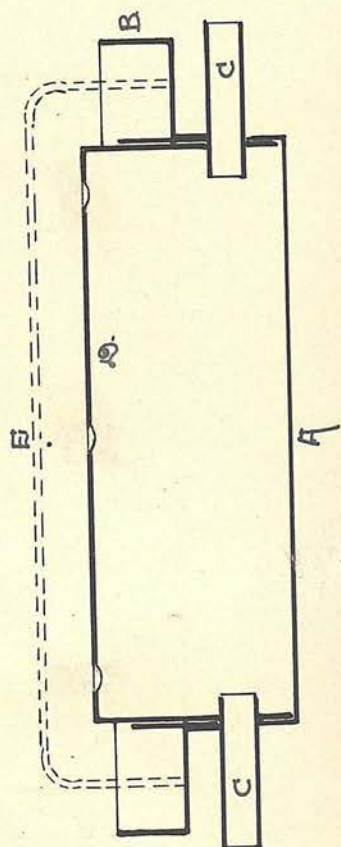
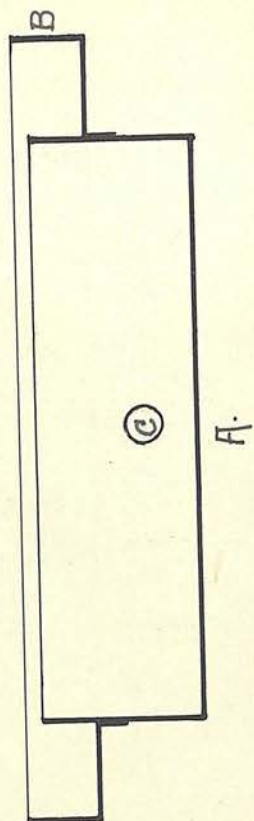
render the lid air-tight by utilising this flange and trough arrangement. This is done by running a little melted paraffin wax into the trough, when the lid is in situ. On solidifying the tin will be found to be hermetically sealed.

By means of the two angled tubes which pass through the lid, air may be replaced by Hydrogen or Pyrogallate of Soda may be drawn in after producing a slight vacuum.

Such a tin serves the same purposes as a Bulloch's apparatus without being so cumbrous or requiring so much incubator space. Further, as such a tin measures 6 inches across by 6 inches deep, there is room for a very considerable number of culture tubes.

2. For Plate Culture. Such an apparatus, however, is not so suitable for plate cultures where space has to be economised, and where one desires to observe the appearance of the growing colonies from day to day. One of the best methods in use so far is that devised by Captain Henry, ^{28/}R.A.M.C.

This is open, however, to one or two objections which seem to be obviated by the use of the apparatus of which I give a description. For instance, the metal part of Henry's apparatus is not sterilised before/



before use, pyrogallate of soda only can be used, while the luting of the 2 ledges takes up some time - a matter of importance where many have to be done.

The apparatus which I use in Reading consists of three parts. The lowermost part, "A" resembles an inverted tin lid made in one piece to the outer side of whose rim has been soldered a right-angled ledge of tin "B". This runs all the way round, and together with part of the vertical rim of "A" forms a trough or fossa.

The diameter of the tin tray is 3 inches, and its depth $\frac{3}{4}$ inch. The breadth of the fossa is $\frac{1}{8}$ th of an inch, and its depth, measured on the inner side, is $\frac{1}{4}$ inch.

Let into the tin tray "A" and soldered in position, are two small tin tubes "C" which project slightly into the interior of the tray and outwards for $\frac{1}{2}$ an inch, so that they do not project beyond the ledge above mentioned.

Attached to each of these is a small piece of rubber tubing controlled by a clip or a piece of inserted glass rod.

The second piece "D" again resembles a tin lid, this time in the uninverted position, and made in such a way as to fit fairly closely into the tin tray "A".
It/

It is provided with two right-angled slits, so that when pushed home into tray "A" the slits coincide with the projecting ends of the two tubes. By giving "D" a partial turn the right-angled slits form a sort of bayonet catch with the inner projecting tubes, and so prevents the 2nd piece "D" from falling apart from "A" when the apparatus is turned upside down. When "A" and "D" are in contact, there is formed a cavity $\frac{1}{8}$ ths of an inch deep, into which pyrogallate of soda may be run.

The top of lid "D" is pierced by a series of small holes and is coloured black so as to form a dark background against which colonies may easily be counted.

The third piece "E" consists of one of the constituent parts of an ordinary Petri dish. That figured in the drawing is a $3\frac{1}{2}$ inch plate.

Before use, some cotton wool, if pyrogallate of soda is to be used, may be placed in the cavity formed by the two pieces "A" and "D" and the whole apparatus - glass and tin - is sterilised together.

For use, the apparatus is inverted so that the glass part is lowermost, and the inoculated culture medium is rapidly poured into it and the tin part replaced.

When/

When the medium is sufficiently set, the apparatus is reversed so that the glass part is now uppermost, and a small quantity of melted paraffin wax is run into the trough so as to form a thin layer at the bottom. When this solidifies, as it does almost immediately, the glass lid will be found firmly fixed to the tin ledge and to be quite air-tight.

The next step is to remove the available Oxygen either by displacing the contained air by Hydrogen or by running in a small quantity of pyrogallate of soda through the tubes "C" provided. Both methods are equally simple, and neither is used till the apparatus is air-tight. Where a Kipp's apparatus is not available, it is a simple matter to keep a stock bottle of pyrogallate of soda made up as recommended by Haldane.²⁹

When the whole apparatus has been put together, its total vertical depth is only $1\frac{1}{4}$ inch.

The width of the ledge is intentional, so that different sizes of Petri dish may be used according to what is available.

It possesses, therefore, the following advantages:-

It can be sterilised with the glass lid in situ in the same way as the ordinary form of Petri dish is sterilised.

The/

The risk of air-borne infection is reduced to a minimum, as the apparatus is only opened for a second while the medium is introduced. It can be hermetically sealed in a few seconds, and is thus most economical of time. Either hydrogen or pyrogallate of soda may be used in the way indicated in order to displace or remove available Oxygen. The technique involves no special skill, and finally the tin parts of the apparatus are cheap, as it can be made for less than a shilling. e/

The apparatus in use here was made by Messrs Huntly, Bourne & Stevens, Reading.

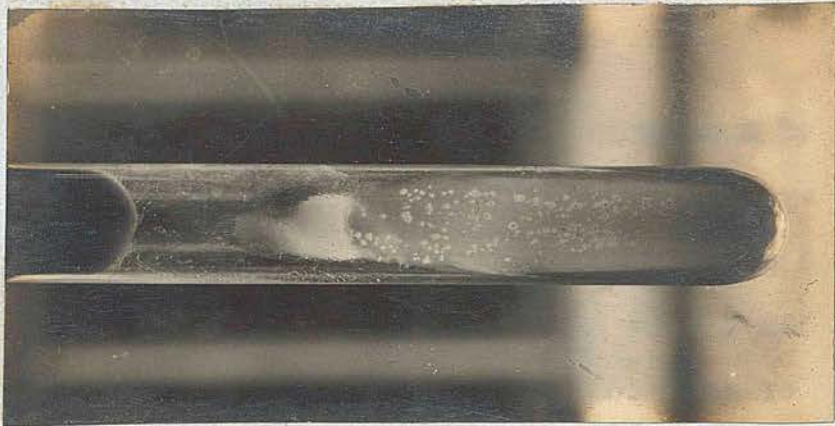
Practically all cultures emit a strong characteristic and distinctly foetid though not unbearable odour. This has already been remarked upon in a previous part of this thesis. It is perhaps best observed in Hirnbrei or meat-broth tubes, and, if the culture has been raised from spores, does not become really strong till towards the end of the second or till the third day. In a week or ten days' time, it begins to diminish somewhat. (Note: By "Meat-broth" tubes, I mean tubes containing broth and minced-up bullock's heart.)

Gas is also formed in greater or less degree, according/

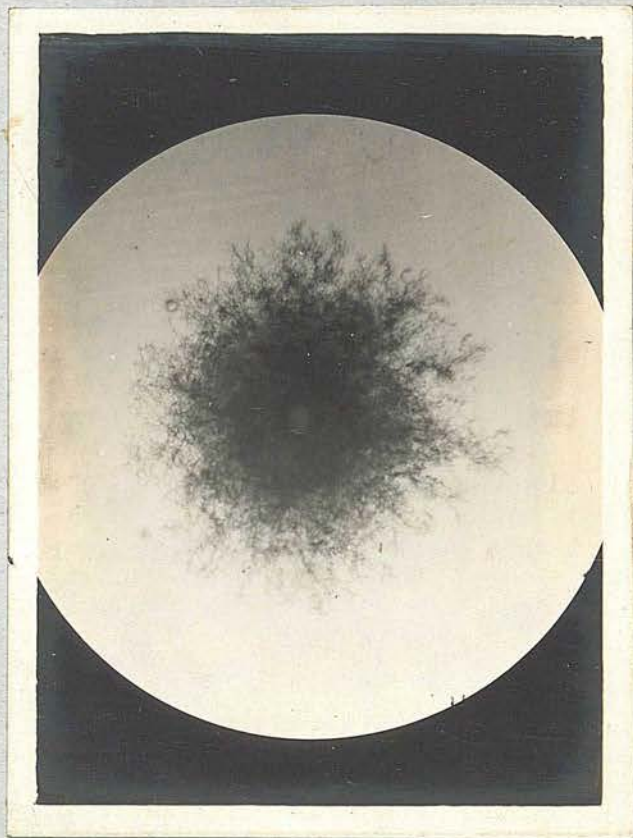
according to the medium employed.

Meat-broth.

In this medium, inoculated from a culture containing bacilli and spores and grown anaerobically at 37° Cent., the supernatant broth, previously clear, is found within 18 hours to have become cloudy. The characteristic odour has begun to be apparent, and the meat, previously of a brownish tint, has now become reddish brown. Within 40 or 48 hours the meat will be seen to have become darker or black in colour, and to have begun to diminish in volume. The particles of meat appear to be going into finer subdivision. Within 60 hours the volume of meat will have become reduced to about half what it was previous to inoculation and incubation, and to be almost quite black. Where spores only are sown in a meat tube, the same sequence of events occurs, except that the production of the characteristic odour and the blackening of the meat take a little longer to make themselves apparent. The diminution in the volume of the meat can be watched and measured from day to day, as well as the gradual diminution in the size of the meat particles. During the first two or three days, gas bubbles can be seen caught in the meshes of the meat particles, and, where the meat broth tube has been rendered anaerobic by means/



1. Photograph of agar slope culture of the Reading Bacillus.



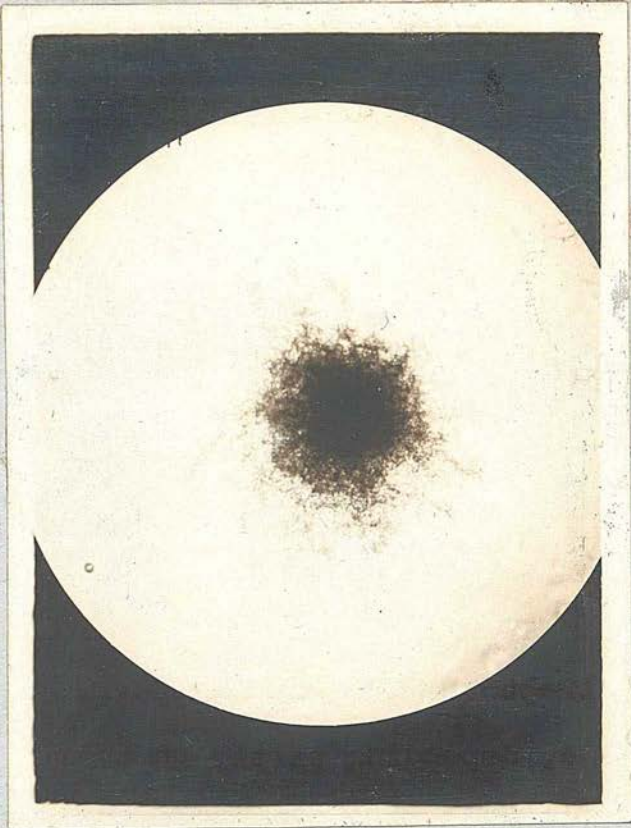
2. Single Colony grown on an agar plate for 18 days. X 10.

means of a supernatant layer of liquid paraffin, the meat may be seen carried up en masse to float on the surface of the broth.

When a meat tube has been left standing on the bench for some considerable time, it will be found that the turbidity disappears from the upper strata of the broth and a whitish, woolly deposit settles on the top of the meat, which contrasts with the black "sloughy" appearance of the remaining meat.

ON AGAR.

Surface colonies on this medium are small, resembling in point of size those formed by streptococci. They are slightly raised, rounded, shiny and translucent at first but later tend to become more opaque. Their edges are not sharply defined, but merge into a radiating tangle of fine thread-like projections for the definition of which a hand-lens is generally required. In place of or associated with these may sometimes be observed rather broader out-growths which look like transparent fimbriae. If the water of condensation has become infected, growth takes place fairly rapidly in the form of a faint, opalescent film spreading/



3. Single Colony grown on an agar plate for 18 days. X 10.



4. Single Colony from an agar plate X 120.

spreading up between the tube and the medium. Such a film frequently shows what appear to be alternations in density, while the free growing edge is generally fimbriated. The agar ultimately becomes fissured and split up by the gas generated.

Deep colonies can just be seen with the naked eye after 48 hours' growth, again similar in size to those produced by streptococci. They appear, however, slightly opaque, depending partly on their depth in the medium and partly on their size, and are of a somewhat dirty white colour. With a hand-lens the central part does not appear to be always of uniform shape but may be oval, triangular, etc., with rather blurred edges which merge into a tangled mass of filaments growing out from it into the surrounding medium. The resulting colony looks somewhat fluffy and resembles a little speck of partially teased out cotton wool embedded in the medium. The amount of growth and the rapidity with which it occurs depends amongst other things on the composition of the medium. The best growth, however, is probably obtained on agar to which glucose or horse-serum has been added.

Micro-photographs have been appended to illustrate some of the points mentioned in relation to the appearance of the colonies on solid media.

Blood/

Blood Agar.

On this medium colonies can be seen within forty-eight hours. In three or four days there is a copious growth partly confluent and partly composed of discrete colonies. The latter are small, more or less rounded flat discs with a slightly elevated node in the centre. This node and the disc on which it is perched have a somewhat shiny appearance. The edge is blurred and merges into a somewhat dry, glazed-looking, feathery or filamentous outgrowth which radiates in every direction and intertwines with the filamentous processes from adjacent colonies.

The confluent part is smooth and glossy, with a suspicion here and there, towards the upper part, of tiny projecting dots, presumably the original discrete colonies which have formed foci from which the confluent growth has taken place. Towards the lower part, these are less noticeable, probably because the confluent growth has there become thicker. The free edge shows either fine, filamentous projections or larger and more pronounced fimbriae.

The colour of the surface growth is greyish-white and stands out in slight contrast with the red background of the medium.

A few bubbles of gas may be seen in the lower part of the tube between the medium and the glass. The water of condensation, if infected, is turbid, but there is no crumbling away of the medium such as is seen in the inspissated serum tubes. The odour is characteristic. With a hand-lens and transmitted light the individual colonies are opaque in the centre corresponding to the central node, less opaque but not quite transparent in the area immediately around this centre, and this in turn merges into transparent slender thread-like processes.

After several days' growth the red colour of the medium will be seen to have become brown. The surface growth has increased, and especially towards the bottom of the slope has a dirty, greyish greenish white tint. Towards the upper part of the slope where the growth is less dense, small moist slightly raised, shiny nodes stand out on a dull glazed background composed of interlacing filaments.

No digestion appears to have taken place.

Ascites Agar.

On slopes made with ascites agar, within twenty-four hours can be seen small but definite colonies. These/

These look like round, shiny domes with blurred edges, from which a tangle of spidery filaments proceeds. Within forty-eight hours, towards the lower part of the slope along the needle track, the growth is confluent and is represented by an opaque, opalescent, whitish film. This streak has a narrow fringe of fine filaments. The agar shows numerous fissures and the water of condensation is turbid. The odour is characteristic.

After a few days' incubation the surface presents a smooth, homogenous, shiny, opalescent film, which is thicker and more opaque towards the lower part of the slope, where it resembles a streak of yellowish white paint irregularly laid on.

No crumbling away has been observed.

Dorset's Egg Medium.

This is an admirable medium on which to obtain a profuse growth of the Reading bacillus.

Within twenty-four hours can be seen a moist, whitish film of growth, sometimes traversed by fissures or cracks, and occasionally showing one or two more prominent areas where growth has been more profuse.

Viewed from its posterior aspect, the medium will be seen to be in process of turning a greenish-black colour, /

colour, while at the bottom of the tube will be noticed a somewhat turbid fluid, perhaps containing fragments of broken-down medium.

The culture emits a very powerful and offensive odour.

In forty-eight hours will be noticed very definite crumbling away of the lowermost part of the Dorset-egg slope. The turbid fluid of liquefaction will be found to have increased in volume and, floating in it, will be seen fragments of egg medium of various sizes, together with oil droplets. The fissures in the surface growth have now disappeared, and the whole is covered by a thick moist creamy growth. Sometimes the growth looks as if half-curdled milk had been dribbled over the surface. Viewed from behind, the medium will be seen to show a greyish-black tint, most marked where the medium is thinnest. This blackening appears to affect all the medium except for a narrow margin all round which still preserves its yellow character. Disorganisation and thinning of the medium proceed rapidly under further incubation. The medium itself becomes split and fissured, and those parts not yet all eaten away sink down in large pieces into the dirty, turbid and disgusting-looking fluid.

Inspissated/

Inspissated Horse Serum.

Within twenty-four hours slight darkening of the opalescent medium will be found to have taken place, slight crumbling away will be seen at the lowermost edge of the slope, together with a small collection of water of liquefaction in the bottom of the tube.

In forty-eight hours the latter can be seen to have increased considerably in volume, and to have become quite turbid. The crumbling away of the medium has increased in extent, and the lowermost part of the slope, especially at the crumbling edges, will be found to be blackish and dirty-looking. The surface growth differs somewhat from that seen on Dorset Egg medium. It is covered with what looks like a film of very closely apposed, minute, more or less rounded, pin-point colonies, practically touching one another at their margins, and only very slightly raised.

They require a hand-lens for their proper appreciation. They have a somewhat dull, glazed appearance, and give the surface a sort of fine, morocco-leather appearance.

The odour is again strong and characteristic. In the course of a few days what remains of the medium is seen to be more translucent than that in an uninoculated tube, and the surface colonies can now be distinguished on looking at them from the posterior aspect/

aspect of the tube.

If left standing undisturbed for some days, a slight deposit of black, pigmented material accumulates at the very bottom of the tube. Over this is the turbid fluid of liquefaction, and on the top a dirty, whitish, flocculent layer. Gas does not appear to be produced so abundantly as in glucose agar tubes.

The extent of disintegration steadily increases with further incubation.

Gelatine.

Following gelatine stab there may be seen within thirty-six to forty-eight hours a faint, gauzy streak along the needle track. A few hours later, small, somewhat globular points can be seen, and from the track of apparent growth will be noticed a few, fine, short lateral offshoots which tend to grow outwards and upwards. A somewhat crateriform excavation occurs at the top of the medium containing a turbid fluid in which a flocculent growth is suspended. The upper part of the medium now becomes entirely fluid, and projecting downwards from this into the solid medium is a nipple-shaped cone of liquefaction. Extending downwards from the apex of this, along the needle track, is a more or less loosely arranged column of small, woolly colonies. Each seems to have a slightly denser central part or node, and from them radiate fine thread-like/

thread-like filaments. Liquefaction appears to be taking place in relation to some of these colonies, so that they look like little clusters composed of small fluid bladders. The whole soon becomes entirely fluid. The liquefied material is turbid, opalescent, and contains woolly particles floating in suspension, together with a more dense deposit of the same flocculent material towards the bottom.

Gelatine seems to be a very satisfactory medium on which to grow this organism. Films made from such a tube show bacilli of all sizes, all very actively motile. Many possess typical spores, and numerous filamentous forms can be seen.

Potato.

No growth has been found visible to the naked eye after several weeks' incubation on this medium. With a hand-lens, however, growth appears to have occurred but to a very slight degree, as one or two small slightly dome-shaped, moist colonies have been seen like little specks of honey.

Nutrient Broth.

After three days' anaerobic cultivation in this medium, the broth will be found to be turbid and to contain small flocculi in suspension. There is a definite, /

definite, woolly deposit at the bottom, which rises up in a cloud on shaking and becomes suspended in the medium.

The odour is characteristic, and a small amount of gas is formed. Films made from this show typical sporing, motile bacilli, as well as non-sporing forms. Filaments may be seen as well as numerous clostridial forms.

On standing for some days, the floculli settle at the bottom in a more or less compact mass, leaving the upper strata of broth fairly clear, except in the case of broth to which glucose has been added. The amount of turbidity, which corresponds more or less closely with the degree of growth, will vary according to the ingredients used in the broth. For instance, the cloudiness is most marked, and most persistent, in glucose broth, and least in glycerine broth. Ordinary nutrient broth occupies an intermediate place.

The amount of gas generated will also depend on the same factors. It is considerable in glucose and in glycerine broths, respectively; somewhat less in plain nutrient broth, and least of all in inulin broth. For instance, we may find that in glucose broth the Durham's tube is half full of gas; in the glycerine broth, one third full; in plain, nutrient broth, one sixth/

sixth full, and in inulin broth only one twelfth full.

Neutral Red Broth.

In strong concentration no change in colour is observed and no growth appears to take place in this medium.

In 5 c.c. of broth to which 0.1 c.c. of a 1% solution of neutral red has been added, growth occurs within twenty-four hours with the production of a yellowish-green fluorescence. Where double this quantity of neutral red has been used, growth still occurs, but the change of colour is not noticed for forty-eight hours.

Fluid Egg Medium.

In this medium, made up as Miss Robertson suggests but without its dilution with broth, growth occurs readily with the production of a powerful and characteristic odour.

Within twenty-four hours gas is generated and some of it can be collected in a Durham's tube. The medium becomes a dirty yellow colour, and quite opaque. It differs in this respect from the uninoculated medium through which the outlines of the Durham tube can fairly easily be seen. After a day or two, a heavy sediment deposits, and the dirty yellow fluid is/

is seen to be full of small granules held in suspension.

Films made show typical sporing and non-sporing motile forms, together with numerous clusters of fine, radiating, needle-like crystals and a good deal of amorphous debris.

Litmus Peptone Water.

In peptone water slight growth occurs after two or three days' incubation, and the water becomes slightly turbid. Where litmus has been added the tint becomes unmistakeably blue.

When a culture in plain peptone water has been allowed to stand undisturbed for a day or two, a slight layer of black pigmented material will be found at the very bottom of the tube and overlapping it and lying superficial to it will be found a layer of greyish, woolly deposit. With an oil immersion, the black material is seen to be amorphous, while the fleecy particles contain large numbers of spores and a few typical bacilli.

No Indol appears to be formed.

Litmus Whole Milk.

Good growth takes place in this medium, a small amount of gas is generated, and a more or less typical odour is produced.

No/

No clot has been observed after many days' incubation at 37° Cent. Instead, the milk appears to become finely granular, much less opaque and more watery. After two days' anaerobic incubation the following is observed. On removal from the tin, the medium is seen to be blanched. On standing for a short time it turns a pink tint which gradually deepens to mauve. The medium is seen to be composed of two more or less equal strata. The lowermost consists of very slightly turbid serum, while the upper is composed of a dense aggregation of fine granules, but there is no trace of clot. On shaking the tube, these granules become uniformly distributed throughout the fluid, which appears more watery and less opaque than the uninoculated milk. The same phenomena will be observed even after many days of incubation.

Litmus Whole Milk with Precipitated Chalk.

In this medium gas is produced in slightly larger amount than in milk containing no chalk. While the same two strata are present, their relative positions are altered. For instance, after three days' anaerobic cultivation at 37° Cent., the Durham's tube may be half full of gas, as compared with only 1/6th or 1/8th in the non-chalk milk. The upper stratum now consists of serum, mauve in colour and slightly turbid. The/

The lower stratum contains a copious, loose, flocculent deposit collected mainly in a mass round the lower part of the Durham's tube. No clotting has ever been observed. On shaking this up, the deposit becomes, as before, uniformly distributed throughout, and the result is a bluish mauve fluid with many fine granules floating in suspension. While the tint is somewhat more blue than in the non-chalk milk, it is nevertheless of slightly pinker character than that seen in an uninoculated milk containing chalk. In this respect, viz., its apparent reaction in milk, the Reading bacillus appears to differ from the type isolated by Miss Robertson from war wounds, and which she regards as identical with the *b. oedematis maligni* of Koch. In the case of the milk inoculated with her organism, it is said to become alkaline. In the case of the Reading bacillus, on the other hand, the milk has always, even weeks after inoculation, remained pink in colour. Of course, where precipitated chalk has been used, this reaction is less marked, but none the less, even here, it tends to remain pinkish. The odour is well marked. Films made from the two types of milk are interesting.

In the case of the chalk milk, films made on the fourth day of incubation show large numbers of typical sporing/

sporing and non-sporing bacilli, many of which are actively motile, many free spores, all typical, and many small, irregularly shaped granules aggregated in clumps.

In non-chalk milk, on the other hand, the bacilli are not so numerous as in the chalk-milk. They are mostly of the sporing type, and only feebly motile. Filamentous forms can also be seen. Nearly every field is crowded with irregularly-shaped granules, which are present in much larger numbers than in the chalk milk. It looks as if the precipitated chalk in the milk neutralised to a large extent the acid formed by the reaction of the organism on the medium, so that not only were the bacilli present in larger numbers, but they had been able to reduce the granular content of the milk to a much greater extent. It looks as if the bacilli, in whole milk at least, produced a slightly acid environment which, although very slight, was sufficient to retard its activities. Where this acid can be neutralised, the activities of the organism go on with less impediment, and the milk becomes more watery.

In films made from both forms of milk, numerous oil globules were present.

Carbohydrate/

Carbohydrate and other Media.

The reaction on these media has been determined on seven different occasions with a similar result each time. Ordinary litmus peptone water and serum litmus agar, each with the appropriate amount of test substance, e.g., sugar, glucoside, etc., have been used in order to determine the changes, if any, induced by the growth of the Reading bacillus. The test substances used were glucose, lactose, maltose, laevulose, dextrine, galactose, sorbite, saccharose, mannite, raffinose, dulcitol, nutrose, inulin, starch, glycerine and salicin.

While these reactions, which have been always consistent, are probably to be regarded as correct, I think it only right to state that the sugars, etc. employed were not pre-war stock, and the peptone used was Fairchild's, so that I cannot say absolutely that in each case the test substance used was pure. I can only give the results based on work done with the materials available.

Media containing glucose and maltose respectively quickly become acid in reaction, while a considerable quantity of gas is generated. Laevulose and dextrine in the same time are also rendered acid, but to a slighter extent, while the amount of gas produced is about/

about the same. Saccharose and sorbite media become lilac in tint, the former becoming later rather more pink than lilac, but evolving less gas.

No definite acidity has been observed in mannite, lactose, raffinose, dulcitol, inulin, galactose, nutrose, starch, glycerine or salicin, except in the last named, where, after ten or twelve days' incubation, the blue colour has been found to give place to a lilac or even a pinkish lilac tint. Of all the test substances employed, therefore, glucose and maltose alone yield a speedy and unmistakeable reaction. Laevulose and dextrine show the same change in the same period, but to a less degree. Sorbite speedily becomes definitely lilac, while saccharose and salicin, if incubated long enough, also tend to become pink. All have a marked odour, but there appear to be slight differences in the different tubes, several of which have an odour resembling that which hangs about a tannery. Gas is formed in all. If the amount caught in the Durham's tube in the case of glucose fill half of the tube, sorbite and glycerine would only yield one third, maltose one fifth, laevulose one seventh, while mannite, raffinose, dulcitol, lactose, saccharose and inulin would be represented by one tenth. The organism, therefore/

therefore, does not appear to possess very marked saccharolytic powers.

As regards the appearance of the organism in films made from the media containing these various test substances, the picture is much the same in the case of glucose, maltose, laevulose and sorbite. In each of these there appears to be a good growth of typical bacilli, most of them having spores. Both short and long, as well as clostridial forms are seen. They are only very feebly motile, and free spores are abundant, many occurring in chains.

In films made from media containing saccharose, the growth is more scanty, and while spores mainly are present, there is a fair number of the bacillary form.

In films made from media containing glycerine, mannite, raffinose, dulcitol, lactose and inulin respectively, the growth is very sparse, and consists almost entirely of free oval spores.

Optimum Reaction for Growth.

To determine this, a series of nutrient broths were put up, covering a fairly wide range of reaction to phenolphthalein, viz., from +50 to -50. These reactions were determined in the cold after sterilisation had been effected. Each of these was sown with a/
a/

a definite and similar amount of an old broth culture which had previously been heated to 80° Cent. for ten minutes.

They were then grown anaerobically for three days at 37° Cent. after which they were examined macroscopically and microscopically. They were then incubated for a further period of five days anaerobically, at the same temperature. After three days' incubation, the following was the result:

Initial reaction	+10.5	+5.5 +3.5	-0.5
Appearance after 3 days' incubation.	Slight turbidity and slight odour.	Very turbid and opaque with a powerful & characteristic odour.	Suspicion of turbidity & odour doubtful.

No change was observed in broths of higher reaction.

After ten days' anaerobic incubation at 37° Cent. definite growth was obtained in broths having an initial reaction to phenolphthalein of +16.75, +10.5 and - 0.5 respectively.

Probably the most suitable reaction, therefore, for obtaining a profuse growth of the organism is about +5 to phenolphthalein.

When the inoculated broths in which growths had occurred/

occurred were titrated in the cold after incubation, they were found to have become slightly more alkaline.

Films made from broth of each reaction after three days' incubation corroborate the macroscopic findings.

In films made from broth of +10.5 reaction, a comparatively small number of bacilli were found. These were for the most part the non-sporing form of the Reading bacillus occurring singly, in pairs, and occasionally in chains. One or two showed commencing spore-formation. The bacillus were motile. In films made from broth of +5.5 reaction, bacilli were present in large numbers. These were for the most part typical Gram-positive, oval-sporing bacilli which in hanging drop were actively motile. A few were without spores, and some showed beginning spore-formation.

In films made from broth of +3.5 reaction, the picture was much the same, except that the number present was obviously not quite so large, and many showed chain-formation. In films made from broth of - 0.5 reaction, the appearance and numbers were similar to what was seen in films made from the broth of +10.5 reaction. While a few had typical spores, the majority were in the non-sporing form. All were actively motile in the hanging drop preparations. Neither spores nor bacilli were observed in films made from/

from any of the other broths, with the exception of that with a reaction of + 16.75, where there were a few non-sporing forms.

Range of Growth.

The organism will grow quite well at 18° Cent., but not so rapidly as at 37° Cent. Attempts have been made to grow it at 41° Cent., but growth here is very slight. No attempt has been made to grow it at intermediate temperatures.

Resistance to Heat.

For this purpose old broth cultures were used in one series of experiments, and for another, a suspension in sterile water of colonies taken from old agar slopes which had been lying at bench temperature for a week at least after removal from the incubator. The reaction of the broth was the ordinary + 10 reaction to phenolphthalein,

In each case the test tube containing the organisms or their spores was immersed in a beaker of water which was kept boiling vigorously. At minute-intervals a measured quantity was removed with a sterile pipette and sown in tubes containing meat broth. These were incubated for many days, being examined at intervals.

In the case of the sterile water suspension, growth stopped after fifty-five minutes' exposure.

In/

In the case of the broth cultures, growth occurred from the sample which had been exposed to water kept at 100° Cent. for fifty-nine minutes, but no growth was obtained after longer exposure.

An old broth culture was then boiled in a test tube over the naked bunsen flame, and kept boiling for some time. At intervals of a minute, measured quantities were removed with a sterile pipette and sown in meat broth tubes. Growth occurred after five minutes boiling, but not after longer exposure.

It will be seen, therefore, that the heat-resistance of the spores in the particular environment tested is very considerable indeed.

Dessication Tests.

These were performed in order to determine how long the spores of the Reading bacillus were capable of remaining alive in the dried condition, so that when suitable conditions were again provided, they would be able to grow out afresh. Test tubes each containing a small pledget of cotton wool were sterilised. Into each were placed three or four drops of an old broth culture of the Reading bacillus. The tubes were then placed for 48 hours in a drying chamber kept at 80° Cent. They were then removed, some were left exposed to the light at bench temperature, and others stored in the cold incubator. Broth was at first/

first added to one of the tubes after it had been kept for eight days in the cold. No growth took place, even after some weeks' incubation. Another tube was treated similarly after a stay of twenty-six days in the cold incubator, and still no growth was obtained. To others, after varying intervals of time, were added meat and broth, when growth occurred normally. Growth has been obtained in this way after a period of 8 months.

These tests go to prove that in its spore-form the Reading bacillus is able to remain alive in a more or less dried condition for a considerable length of time. Exactly how long has not yet been determined. Taken in conjunction with its resistance to heat and its ability to grow at comparatively low temperature, the above results seem to indicate that, in a possible habitat like the soil, the Reading organism may retain its vitality for considerable periods in spite of climatic variations.

They suggest, however, that this property might be turned to advantage in distributing the organism for treatment purposes. As a vehicle for the transmission of these spores, sphagnum moss seems peculiarly suitable. The latter, moreover, as I have pointed out, forms an excellent packing for wounds. It is composed of countless microscopic chambers, communicating at intervals/

intervals with the outside by means of minute pores, and prevented from collapsing by its skeleton of pectin, the whole forming a most efficient sponge. In broth, sphagnum moss plays to a certain extent the same part as do the pieces of fresh tissue recommended by Tarozzi. Fragments of moss removed from a culture tube containing Reading bacilli and examined under the microscope, reveal large numbers of the living organism packed within these chambers. Moss so impregnated with the bacilli or their spores could be dried and prepared ready for placing directly in the wound, which would thus at the same time be inoculated.

Growth in Relation to Various Concentrations of Salt.

A series of experiments were carried out to determine the limiting concentration of salt beyond which the Reading bacillus would not grow, and at the same time to find out if it was necessary for growth that the salt should be present in strong concentration in the wounds, as it undoubtedly appears to be for some hours at least after its introduction.

In the first instance varying percentages of salt were made in glucose broth by adding to each tube containing 5 c.c. of sterile broth, the appropriate amount of salt. These tubes were then sterilised in the steamer on three successive days in the usual way.

The percentages refer to the amounts of salt weighed out for each tube and of course are not absolutely accurate inasmuch as no account has been taken of the water of crystallisation or of the increase/

increase of weight due to the hygroscopic properties of the salt. The error, so far as this work goes however, is negligible. To each of these tubes a measured quantity of an old culture grown in meat broth was added and the series incubated at 37° cent. for eight days. The results are set out in the accompanying table:-

Salt Con- centration	5%	10%	15%	20%	25%	Saturated.
Growth.	+	-	-	-	-	-

A second series of glucose broths containing varying percentages up to 10% were next put up, and examined in the same way. These are shown in the next table:-

Salt Con- centration	0	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%.
Growth.	+	+	+	+	+	+	?	-	-	-	-

Growth was strongest in the broth to which no salt had been added. Tubes 1 to 5 showed the presence of the non-sporing form of the bacillus, but the numbers diminished gradually as the salt concentration increased till, in the tube containing a 6% concentration of salt, spores only were present. In the other concentrations no growth occurred. The Reading bacillus or its spore-form had not been killed, however/

however, by contact with the higher concentrations, for subcultures, made into meat broth from all the tubes, yielded on incubation a typical growth of the bacillus.

A third experiment was carried out in which plain nutrient broth was substituted for the glucose medium, and with salt concentrations ranging from 4 to 8%. Each tube was sown from an old sporing culture and incubated anaerobically at 37° cent for 6 days. The result is shown in this table:-

Salt Concentration.	4%	5%	6%	7%	8%
Growth.	+	+	-	-	-

A fourth series was investigated to determine how certain other organisms would behave in relation to various concentrations of salt. In this particular experiment, the appropriate quantities of salt were first weighed out into test tubes, which were then sterilised. To each of these were added 5 c.c. of sterile peptone water. It was found, however, that the peptone apparently "salted out" and this particular method of obtaining sterile salt concentrations was not again used.

The results, however, are given in the accompanying table. Two fresh strains of staphylococcus aureus and/

and two of streptococcus longus were used and after inoculation the tubes were incubated for 24 hours at 37° cent.

Salt Concentration.	5%	10%	15%	20%
Growth of Staph Aur. I.	+	Slight	-	-
" " " " II.	+	Slight	-	-
" " Strep. Long. I.	-	-	-	-
" " " " II.	-	-	-	-

A fifth set of cultures was next examined, the culture tubes containing nutrient broth, each with its proper percentage of salt. These were sterilised in the steamer on three successive days after the addition of the salt - a method by which "salting out" is, for the most part, avoided.

The organisms investigated on this occasion were staphylococcus aureus, streptococcus longus, bacillus pyocyaneus and b. coliformis.. The results were as follows:-

Salt Concentration	1%	5%	10%
Growth of Staph. Aur.	+	+	+
" " Strep. Long.	+	+	-
" " b. Pyocyaneus	+	+	-
" " b. Coliformis	+	+	-

In this series, growth, as determined by opacity of the medium and by the examination of films, was more vigorous in 1% concentrations than in 5% and, where growth occurred, in 10% as compared with that in/

in 5%. From a consideration of these experiments it would appear that the Reading bacillus does not proliferate in nutrient broth where the salt is present in greater concentration than 5%. In this percentage it will grow but not so vigorously as in lower concentrations. In salt concentrations higher than 5% only free spores were found, but although growth was inhibited, meat broth tubes, inoculated with a sample from each of these, showed evidence of growth in all the tubes as far as that sown from the 15% salt concentration. Beyond this, growth did not occur, i.e. in meat broth tubes inoculated from the 20%, 25% and saturated salt concentrations respectively.

This seems to point to the fact that if the salt in salt-bag packed wounds could be maintained at its initial concentration, the growth of the Reading bacillus would almost certainly be inhibited. Salt, in this high concentration, probably delays the proliferation of the bacillus when present, and the latter only begins to become active as the salt concentration becomes reduced. This reduction is probably effected in large extent during the first 24 hours by the copious outflow of fluid from the wound, carrying away in solution much of the salt into the superficial cotton-wool coverings. Girling Ball has investigated the content of salt packs after the latter have been in/

in situ for four days. After soaking such salt packs and expressing the fluid contained in them, he has only been able to recover 2% of Salt. This seems to show that in four days a very considerable reduction must have taken place in the original salt concentration. These experiments and observations tend to show that a high salt concentration is not only unnecessary ~~but~~ but, if it were maintained at that high strength, that it would absolutely inhibit the growth of the Reading bacillus. Confirmation of the former statement has already been provided by the expedient of substituting sphagnum moss for salt bags, and observing that equally good results could be obtained by the former method of treatment.

As regards the effect of salt-concentration on certain other organisms, it will be seen again that a concentration greater than 5% will inhibit further growth, except in the case of staphylococcus aureus, which will grow even in a 10% concentration although less vigorously. This, however, presupposes that the salt is maintained at its high initial concentration. The above facts, however, show that this latter is not so maintained but rapidly diminishes, and, if further proof were needed, it is to be found in the statement already made that pyogenic organisms appear /

appear to be as plentiful in films and in cultures made from the pus, both during and after treatment with salt bags, as before such treatment was commenced. Any possible bactericidal or inhibitive effect that salt in high concentration may have, rapidly disappears, probably within a few hours, where the salt-bag method of treatment is employed. The latter, therefore, even if I had no other explanation to offer for the beneficial effects achieved by it, would not depend on any germicidal or inhibitive action on the part of the salt present.

Behaviour of the Reading bacillus towards certain antiseptics.

In order to find out to what extent the Reading bacillus was tolerant of the presence of antiseptics, the following were chosen, viz., acriflavine, phenol, mercuric iodide, eusol, and Dakin's solution. These were put up in certain proportions each in 10 c.c. of broth.

In the case of the first series examined, glucose broth was used as the vehicle. Each tube was sown with an equal amount of a mixed broth culture, containing bacilli and spores of the Reading bacillus, and thereafter incubated for 48 hours. The results are set out in the following tables.

Table 1./

Table I. - Acriflavine.

% in glucose broth of a 1 in 1000 dilu- tion of acriflavine	.1%	.5%	1%	5%	10%
Growth after 48 hours' anaerobic incubation	+	-	-	-	-

Films made from the tube showing growth showed the normal characters of the bacillus.

Table II. - Phenol.

% in glucose broth of a 1 in 20 dilution of phenol.	.2%	1%	2%	10%	20%
Growth after 48 hours' anaerobic incubation	+	-	-	-	-

Films made from the first of these tubes showed a typical growth of the Reading bacillus, which, however, was sporing freely.

Table III. - Mercuric Iodide.

% in glucose broth of a 1 in 1000 dilution of mercuric iodide.	.2%	1%	2%	10%	20%
Growth after 48 hours' anaerobic incubation.	+	-	-	-	-

In the first tube the growth appeared typical but very few spores were seen.

Table IV. - Eusol.

% in glucose broth. of Eusol undiluted.	.2%	1%	2%	10%	20%
Growth after 48 hours' anaerobic incubation.	+	+	+	+	+

Films made from all these tubes showed the typical organism.

Table V. - Dakin's Solution.

% in ordinary broth of the undiluted Dakin's solution.	.2%	1%	2%	10%	20%
Growth after 48 hours' anaerobic incubation.	+	+	+	+	+

In films made from each of these tubes, the bacillus was found in its typical form.

Of all these five antiseptics, therefore, the Reading bacillus appeared to be most tolerant of Eusol and of Dakin's solution.

In the second series further experiments were made into the resistance of the Reading bacillus to acriflavine. The results are set forth in the following tables:-

Table I./

Table I. Tubes sown from the growth obtained in tube 1 of Table 1 in the preceding series, i.e., from the tube containing 0.1% of 1 in 1000 acriflavine in which growth occurred.

% in ordinary broth of 1 in 1000 acriflavine.	.1%	.2%	.4%	.6%	.8%	1%
Growth after 48 hours' anaerobic incubation.	+	+	-	-	-	-

Table II. Ordinary broths containing acriflavine sown from the 2nd tube shown in the last table.

% in ordinary broth of 1 in 1000 acriflavine.	.2%	.3%	.4%	.5%	.6%
Growth after 48 hours' anaerobic incubation.	+	+	+	-	-

Table III. Ordinary broths containing acriflavine sown from the 3rd tube of Table II.

% in ordinary broth of 1 in 1000 acriflavine.	.3%	.4%	.5%	.6%	.7%	.8%	.9%	1%
Growth after 48 hours' anaerobic incubation.	+	+	+	+	-	-	-	-

Table IV. Ditto - but sown from 4th tube in Table III.

% in ordinary broth of 1 in 1000 acriflavine.	.5%	.6%	.7%	.8%	.9%	1%
Growth after 48 hours' anaerobic incubation.	+	+	+	+	-	-

Table V. Ditto - sown from 4th tube in Table IV.

% in ordinary broth of 1 in 1000 acriflavine.	.7%	.8%	.9%	1%	1.1%	1.2%	1.3%
Growth after 48 hours' anaerobic incubation.	+	+	+	+	-	-	-

Table VI. Ditto - sown from 4th tube of Table V.

% in ordinary broth of 1 in 1000 acriflavine.	.9%	1%	1.1%	1.2%	1.3%	1.4%
Growth after 96 hours' anaerobic incubation.	+	+	+	+	-	-

Table VII. Ditto - sown from 4th tube of Table VI.

% in gelatine of 1 in 1000 acriflavine.	1.1%	1.2%	1.3%	1.4%	1.5%	1.6%	1.7%
Growth after 96 hours' anaerobic incubation.	+	+	+	+	+	+	-

Table VIII.

% of 1 in 1000 acriflavine in normal broth sown from a broth culture.	.2%	.4%	.6%	.8%	1%
Growth after 12 days continuous anaerobic incubation.	+	+	+	-	-

Cultures were then made directly from the 3rd tube of Table VIII, into broth containing .8% and 1.2% respectively, /

respectively, of a 1 in 1000 dilution of acriflavine. While growth occurred in the 0.8% dilution, the 1.2% solution remained sterile, even after 10 days' incubation.

A study of the foregoing tables seemed to suggest that where the Reading bacillus is exposed to the action of acriflavine in broth, growth takes place only in a very weak concentration of the antiseptic. As stated above, even after further incubation, the organism only managed to grow in very slightly stronger concentration, the highest point reached being 0.6% of a 1 in 1000 solution.

On the other hand, it looked as if the Reading bacillus could be accustomed to grow in gradually increasing concentration of the antiseptic. By sub-culturing from the highest concentration in which growth had occurred in one series to a tube containing the concentration just above it in which the organism had previously failed to grow, it was thought that a strain more resistant to the antiseptic could be evolved. It certainly looked as if the powers of resistance of the organism grown in gradually increasing concentrations of the antiseptic were greater than those of a strain which had not been exposed/

exposed to the action of acriflavine at all. Such a thing would have a very important bearing on the antiseptic treatment of wounds, for it would mean that a strain of organism, resistant to the antiseptic employed, might be evolved under the continued application of that antiseptic. Indeed, it would constitute an argument for frequent change of antiseptic. When, however, similar experiments were repeated, it was found that there was a possible fallacy in the first set of experiments in that there was no control over the amount of culture used for sowing in each case. In other words, when exactly the same number of organisms was sown in each case, a concentration was reached beyond which the organism could not apparently be made to grow.

Up to a certain point, the growth in any given concentration of antiseptic appeared to depend on the number of organisms sown.

In order to demonstrate this, another series of experiments were conducted on the following lines.

Five sets of culture tubes were used, each set consisting of 10 tubes. Each of the 10 tubes in a set contained varying percentages of 1 in 1000 acriflavine in sterile broth, the amounts ranging from/

from 0.1% in the first tube to 1% in the last, so that each tube contained 0.1% more of the antiseptic than did the tube immediately below it in the series.

A suspension was then made in sterile saline from young agar slope cultures of the Reading bacillus and the number of organisms per c.c. was counted. This suspension was next diluted so that it contained 250 millions approximately per c.c.

Each tube of a set of 10 received the same volume of bacillary suspension, but the amount of the latter was varied for each set. Expressed in terms of number of organisms added, each tube in set A received 125 millions, in set B 87.5 millions, in set C 62.5 millions, in set D 25 millions, in set E 6.75 millions. The tubes were then incubated anaerobically for 21 days, observations being made each week.

The results at the end of this period of incubation are shown in the accompanying table, where growth is represented by a + sign

Table 9./

Strength of Acri- flavine	0.1%	.2%	.3%	.4%	.5%	.6%	.7%	.8%	.9%	1%
A	+	+	+	+	+	-	-	-	-	-
B	+	+	+	+	-	-	-	-	-	-
C	+	+	+	+	-	-	-	-	-	-
D	+	+	+	-	-	-	-	-	-	-
E	+	+	+	-	-	-	-	-	-	-

Sub-culture was then made into meat broth from each of the tubes in which no visible growth had occurred. These sub-cultures were then incubated anaerobically for 21 days at 37° Cent. At the end of this time growth was found to have occurred in set A in the meat broth inoculated from the 1% Flavine, in set B from the 0.9% flavine, in set C from the 1% flavine, in set D from the 0.9% flavine and in set E from the 1% flavine.

These results seem to show that, up to a certain point at least, larger amounts of acriflavine must be used to prevent growth according as the number of organisms sown is increased. They show further that while growth of the bacillus may be inhibited, the spores are not killed even after 21 days' exposure to 1 in 100,000 acriflavine.

A/

A third series of experiments was carried out to determine the behaviour of the Reading bacillus in higher concentrations of Eusol.

The experiment in Table IV of the first series was repeated with a similar result, viz., that the bacillus was found to grow in 20% Eusol.

The Eusol and Dakin's solutions were freshly made up and added to 5 c.c. of broth, so as to yield different percentages. The tubes were then sown from the growth obtained in the tubes containing 20% of Eusol and of Dakin's solution respectively.

The results are shown in the subjoined table:-

Table I.

% of Eusol and of Dakin's solution respectively in 5 c.c. broth	25%	30%	40%	80%
Growth in Eusol tubes after three days.	-	-	-	-
Growth in Dakin's solution after three days.	-	-	-	-

Table II./

Table II. Tubes sown from the growths obtained in 29% Eusol and Dakin's solution respectively.

% of Eusol and of Dakin's solution respectively in 5 c.c. broth.	22%	24%	26%
Growth in Eusol and Dakin tubes after 48 hours' incubation.	-	-	-
Growth in Eusol after 12 days	+	+	+
Growth in Dakin after 12 days.	+	-	-

As considerable precipitate was thrown down in ordinary broth tubes by the addition of higher concentrations of Eusol, four other types of media were employed and Eusol added to make the dilutions recorded in the next table.

Table III.

% of Eusol in 5 c.c. of each of the following media.	28%	30%	Incubated for 96 hours.
Gelatine	+	+	
Glycerine Broth	+	+	
Glucose peptone water	+	+	
Inulin broth	+	+	

The best growth and the least precipitate due to the presence of Eusol was obtained in the gelatine tube./

tube. After prolonged incubation, growth has been obtained in concentrations of 36%. Once again these experiments do not show any acquisition of tolerance on the part of the organism, to Eusol or to Dakin's solution, and this is what one would expect, having regard to the instability of these two solutions. While negative results are obtained after a comparatively short period, further incubation is followed by a definite growth, probably because the power of the antiseptic has become considerably diminished.

I have endeavoured in this section to show to what extent the Reading bacillus is able to grow in the presence of certain antiseptics when added to broth and certain other media. It does not follow that this same action would be exerted by the antiseptic when applied to gun-shot wounds themselves, but they furnish some information as to the resistive power of the Reading bacillus in a certain environment where minute quantities only of the antiseptic are used. They also seem to point to the possibility that the inhibitory effect of an antiseptic like Acriflavine will depend to a certain extent on the concentration of antiseptic used and the mass infection present, while in the case of antiseptics like Eusol their action/

action is more or less of a transitory character. If, however, the concentration of Eusol or of Dakin's solution can be maintained by frequent application, growth of the Reading bacillus will probably be inhibited to a large extent. How far this is possible in a sloughing wound is another question, which is partly answered by a study of some of the clinical cases so treated.

Substances produced by the Reading bacillus in the course of its growth in meat broth.

Large quantities of gas are liberated in the course of its attack on the meat, etc., present in meat broth tubes as has already been indicated. These consist, for the most part, of carbonic acid gas, ammonia and hydrogen sulphide. The latter, probably in the presence of iron derived from constituents in the culture medium, forming a sulphur compound, is sufficient to account for the blackening of the meat. All three gases are easily collected and demonstrated. Skatol is also produced as a result of its proteolytic action, but I have not been able to prove the presence either of indol or mercaptanes. The above, however, are quite capable of explaining its powerful odour.

Proteins/

Proteins, as I have indicated elsewhere, appear to be split up finally into amino-acids, and in certain of the media, egg, for example, many bundles of fine needle-like crystals are formed resembling tyrosin. What other products of decomposition may be formed, will probably require an expert chemist to determine with certainty. I think it unlikely, however, that the products of its action on any sugars present, are in any way concerned with its beneficial action when present in wounds. As already indicated its action is mainly a proteolytic, not a saccharolytic one. It grows equally well and vigorously on meat broth cultures that have been thoroughly dialysed. The results of examination of the flora of war wounds and the evidence so far adduced as to the effect of the Reading bacillus on other organisms in symbiosis with it scarcely warrant me in supposing that the Reading bacillus is likely to give rise to organic acids either in such quantity or of such a kind as to inhibit or influence the growth of the pathogenic organisms which may be present along with it. An explanation of its beneficial action must be looked for in some other direction, and this is probably to be found in the production of an enzyme or enzymes which are more fully discussed later in the course of this thesis.

/c

Question of Origin of the Reading Bacillus.

As the anaerobes generally found in wounds are believed by many to come from faecal contamination, I took the opportunity to examine some of the specimens of faeces sent in, mostly from convalescent dysenteric patients. Thirty specimens were examined from 24 men. The method adopted was to take a sample about the size of a pea, emulsify it in broth and add this to a meat broth tube. In most cases the broth suspension was heated to 80° Cent. for 10 minutes before adding it to the meat broth. In others it was added to the meat broth direct. These were incubated anaerobically, being examined at intervals till there was no likelihood of the organism making its appearance. All remained negative except one specimen received from a patient suffering from ankylostomiasis. The bacillus recovered from this man's faeces showed all the morphological and cultural characters, which belong to the Reading bacillus.

This is certainly a very small proportion from which to recover the bacillus. It is all the more surprising in that *b. sporogenes*, with which the Reading bacillus appears to be most nearly related, was obtained by Metchnikoff both from healthy faeces and/

and from diarrhoeic stools, although there were slight morphological differences between his two types of organism. If it is a more frequent inhabitant of the human intestines, my failure to recover it more often may be due to the fact that the specimens examined were not normal or that the method employed to recover it was faulty. On the other hand it may be a common saprophyte, not of the human intestine, but of that of some of the lower animals.

Having failed in this direction, I secured two specimens of soil, one from the hospital grounds and one from a garden some miles away. The samples were well shaken up separately in sterile water and allowed to settle. From each deposit 0.5. c.c. was pipetted off into tubes of meat broth which were then incubated. The meat in each case became intensely black. The supernatant fluid was then pipetted off from each and placed in water maintained at boiling heat for 40 minutes. Samples of these were then plated out and repeated platings made till colonies were obtained having the characters of those of the Reading bacillus. The organisms so obtained were morphologically the same, blackened and proteolysed meat in the same way, and emitted the same characteristic odour as the Reading bacillus. The colonies obtained from the hospital soil were much more numerous than those got from the garden soil.

While/

While the organisms so obtained have not yet been submitted to the sugar and other tests, there seems every reason to believe that they are closely related, if not identical with the Reading bacillus.

These facts seem to point to the soil as the habitat of this organism rather than to the intestine of man, and the fact that most of the gun-shot wounds have been soil-contaminated lends colour to this view.

Pathogenicity.

That it is non-pathogenic to man when sown even in large amounts in open wounds has been already amply proved in the Reading War Hospital where in some of the wards it is now used as a routine treatment. That it is also non-athogenic to animals has also been mentioned. In the case of the latter the test of its non-pathogenicity has been a severer one inasmuch as the inoculations were made not on an open surface but into/

into the tissues themselves. Unfortunately animal experiment has been greatly hampered by lack of suitable animals and until a sufficient supply can be obtained, much of this work must be postponed. The following observations, however, have been made. They do no more than indicate that probably along these lines a true explanation of the beneficial effects observed may be found.

A. Experimental Inoculation with Reading Bacillus alone.

1. Into Rabbits.

<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>
1. 930 grams	3.5 cc. of saline suspension of a 3 day ascites agar culture.	Intravenously.
2. 2000 grams	6 cc. of fluid from a 4 day culture in meat broth.	Intravenously.
3. 695 grams	3 cc. of a 14 hour culture in meat broth.	Intraperitoneally.
4. 362 grams	1 cc. of a 2 day culture in meat broth.	Intraperitoneally.
5. Unrecorded	2 cc. of a 5 day culture in meat broth.	Intraperitoneally.
6. 940 grams	2.5 cc. suspension in broth of an 8 day ascites agar culture.	Subcutaneously under skin of abdomen.
7. 1075 grams	1.5 cc. of a 2 day culture in meat broth.	Into thigh muscles.

Weight.	Material.	Route.
8. 640 grams	2 cc. of a 2 day old culture in ascites fluid.	Into thigh muscles.
9. 2000 grams	2 cc. of ditto & 1 cc. of fragments of sterile bullock's heart.	Into thigh muscles.
10. 1642 grams	6 cc. of 4 day meat broth culture.	Into thigh muscles.

2. Guinea Pigs.

1. 1000 grams	3 cc. of a suspension in saline of a 3 day ascites agar culture.	Intraperitoneally.
2. 800 grams	3 cc. of a 14 hour old culture in meat broth.	Intraperitoneally.
3. 880 grams	3 cc. of saline suspension of a 3 day old ascites agar culture.	Subcutaneously under skin of abdomen.
4. 335 grams	3 cc. of 5 day old culture in meat broth.	Ditto.

3. Mice.

1.	0.5 cc. saline suspension of 3 day old agar culture.	Intra-peritoneally.
2.	1 cc. ditto.	Ditto.

Results.

None of the above animals showed any untoward sign/

sign or local lesion and all were alive weeks after their inoculation.

Three other batches, of six mice each, were inoculated on three separate occasions with 3-day old meat broth cultures of the Reading bacillus. Amounts varying from 0.1 to 1 c.c. were introduced, into some subcutaneously, into other intraperitoneally. None of the mice in two of these batches showed any ill effect whatever. They all survived and remained alive as long as did the non-inoculated mice used as controls. By accident, however, the third batch, including a few uninoculated controls, were left in a small unventilated box which had been placed unwittingly in such a position as to be exposed to excessive heat. Both the infected and the non-infected animals were discovered some hours later in a dying condition. This accidental occurrence, however, enabled me to make a post-mortem examination of each, and furnished an opportunity of observing whether any pathological changes had been wrought by the Reading bacillus. No lesion, however, was determinable either in the subcutaneous tissues, the peritoneal cavity or the organs, so far as macroscopic examination went.

Cultures/

Cultures were also made from the site of injection, and from the heart blood in each case. The Reading bacillus was recovered from the former in every case, but only from the heart blood of those mice which had been inoculated intraperitoneally.

As regards the cell content of the peritoneal cavity in the case of mice inoculated by this route, what appeared to be endothelial cells predominated, and along with them was a smaller number of the polymorphonuclear type. In one case, however, the cells consisted mainly of lymphocytes and endothelials.

These findings, so far as they go, agree fairly closely with the more accurate results about to be recorded which were obtained from animals inoculated for the set purpose of observing the resulting cytological response. There was, therefore, no evidence forthcoming as a result of the above inoculations that the Reading bacillus was pathogenic to mice, guinea-pigs or to rabbits, nor was there evidence of any toxin present in the cultures employed. To test this point further, however, a small guinea-pig was inoculated intraperitoneally with 11 c.c. of a filtrate from a 6-day old culture of the Reading bacillus/

bacillus grown in meat broth. The dose was rather massive but the animal showed no ill-effects and was alive and well weeks afterwards. In contrast with this is the lethal effect produced on a guinea-pig by filtrate from a culture of *b. perfringens* to which reference is made further on in the text.

Cell Response to Injections of the Reading Bacillus.

To find out whether the injection of living cultures into the tissues is followed by a leucocytosis or not, two rabbits were inoculated, one intravenously and one into the muscles of the thigh with 6 cc. each of/

of a meat broth culture of the Reading bacillus grown for 3 days anaerobically.

The leucocyte counts are shown in the subjoined columns:-

<u>Intravenous Rabbit</u> (male).		<u>Intra-muscular Rabbit (female)</u>			
<u>Before Inoculation.</u>					
1st day	3 p.m.	9,375 per cmm.		6,250 per ccm.	
2nd	" 9 a.m.	17,500	"	12,500	" "
"	" 5 p.m.	15,625	"	13,125	" "
3rd	" 10 a.m.	13,125	"	17,500	" "
"	" 3 p.m.	15,000	"	9,375	" "
<u>After Inoculation.</u>					
"	" 6 p.m.	4,687 per cmm.		6,875 per ccm.	
"	" 10 p.m.	6,250	"	15,625	" "
4th	" 9 a.m.	22,500	"	10,000	" "
"	" 3 p.m.	14,375	"	12,500	" "
"	" 10 p.m.	14,375	"	21,250	" "
5th	" 9 p.m.	21,250	"	12,500	" "
6th	" 10 a.m.	21,375	"	14,062	" "
"	" 3 p.m.	15,000	"	13,125	" "
"	" 9 p.m.	15,000	"	16,250	" "
7th	" 10 a.m.	23,125	"	17,500	" "

It will be seen that at the end of 3 hours there was a drop in the number of leucocytes in the peripheral blood. In the case of the intravenous rabbit, it/

it was very considerable and much more marked than in the case of the intramuscular rabbit. The numbers rose to a somewhat higher level than had been found previous to inoculation, in the case of the intravenous rabbit within 15 hours and in the case of the other in about 28 hours. No account was taken of the influence of feeding on the leucocyte count and it was impossible to make the counts at exactly the same time each day. Such as they are, however, they tend to show that a slight leucocytosis occurred in each case after a fall, more quickly in the case of the intravenous than in the intramuscular rabbit. How far this increase ought to be attributed to the medium in which the organism was grown, I cannot say.

To obviate the disturbing influence of the meat broth medium and to find out what happened when the organism was introduced into the peritoneal cavity, I made a thick suspension of agar slope cultures in sterile saline and inoculated several mice intraperitoneally. The dose given in nearly all cases was 0.5 cc. and the animals were killed by chloroform at intervals. The peritoneal cavity was opened and any fluid present pipetted off and films made therefrom.

The object of examining such films was to find out, not so much the characters of the various types of/

of cell present as to learn whether there was a polymorphonuclear leucocytosis. The results are shown below:-

<u>Time after Inoculation</u>	<u>Bacilli.</u>	<u>Polymorph. Leucocytes</u>	<u>Mononuclear cells.</u>
1 hour	Large nos. free.	23%	77% mainly lymphocytes.
2½ hours	Large nos. free & with- in leucoc- ytes.	86%	14% "
4 "	Ditto	80%	20% "
5 "	"	74%	26% "
7 "	"	65%	35%
8 "	Numbers much less, mainly showing spores, many within leucocytes.	70%	30%
9 "	Practically no organisms seen	52%	48%
19 "	No organisms seen.	38%	65% Of these 47% are lymphocytes & 53% large endothelial cells, many of them show- ing included polymorphs.
23 "	Ditto.	45%	55% Of these 12% are lymphocytes & 43% endo- thelials as before.
24 "	No organism seen.	44%	55% Of these 25% are lympho- cytes & 31% endothelial cells as before.

43 hours.	No organism seen.	30%	70%
			Of these 33% are lymphocytes and 37% endo- thelial cells, some of which still show phagocytosis of polymorphs.

These results appear to indicate that on the introduction of the organism into the normal healthy peritoneal cavity, a polymorphonuclear leucocytosis is induced. This begins to be apparent at the end of the first hour and in two or three hours is very marked. The bacilli, at first plentiful as a result of the injection, do not appear to proliferate to any extent but are phagocyted by the polymorphs present. Those which for the time being escape this fate, apparently begin to develop spores. In about 8 hours, the most of them have disappeared and the numbers of polymorphs have commenced to diminish, and they go on doing so gradually till after 43 hours the number approximates to that found at the end of one hour after injection. Co-temperaneous with this decline in the number of polymorphs, an increase takes place in the numbers of the mononuclear series. Nineteen hours after injection, the larger number of mononuclears consists of endothelial cells which are actively phagocytting the polymorphs. The endothelial cells very/

very gradually diminish thereafter, but are still numerous after 43 hours. Coincident with their gradual disappearance there is a rise in the numbers of lymphocytes present.

Instead, therefore, of proliferating in the living peritoneal cavity, the organisms appear to be rapidly attacked by the polymorphonuclear leucocytes, which gradually ingest them and some at least of the polymorphs are in turn themselves taken up by the endothelial cells, which later make their appearance. There is, therefore, direct evidence that the Reading bacillus exerts a chemico-tactic influence on the polymorphonuclear leucocytes.

Its Relation to other Symbiotic Organisms.

This series of experiments was undertaken to determine the effect, if any, of the Reading bacillus on other organisms grown in symbiosis with it, in the hope of shedding some light on the means by which it exerts its beneficial action.

In the first series, *staphylococcus aureus*, *streptococcus longus*, *b. coliformis* and *b. pyocyaneus* were observed, each of which was inoculated into six meat broth tubes. Three of each set were inoculated also/

also with an equal quantity of a meat broth culture of the Reading bacillus. Two of each, one with and the other without the Reading bacillus, were incubated anaerobically, two similar aerobically and the remaining two of each, aerobically and anaerobically on alternate days. At the end of a certain period, agar slopes were inoculated from each and incubated aerobically at 37° Cent. The results are set out on page 239.

In the second series bacillus typhosus, b. paratyphosus B, b. dysenteriae (Shiga) and b. dysenteriae (Flexner), were observed in relation to the symbiotic presence of the Reading bacillus. In this series of experiments, attempts were made to obtain comparative results by plating out from the broth cultures every two days. The amount plated out on each occasion was 1/100,000th of a c.c. of the broth culture, and the resulting colonies were counted. These results are recorded in the following tables, together with notes on the relative proportion of each kind of organisms, as judged from films made from the broth tubes at the end of culture.

In the matter of enumerating the colonies growing on the various plates I received considerable help from my assistant Dr. McLean so that there was a double check on the figures about to be given.

	Continuous Anaerobic.		Intermittent Anaerobic.		Continuous Aerobic.	
	+R.B.	-R.B.	+R.B.	-R.B.	+R.B.	-R.B.
<i>Staphylococcus aureus.</i>						
After 12 days incubation at 37° cent.	++	++	*+++	*+++	++	+++
After 30 days ditto.	+++	+++	*+++	*+++	+++	+++
<i>Streptococcus Longus.</i>						
After 12 days	-	++	*-	*-	-	-
After 30 days.	-	-	*-	*-	-	-
<i>B. Coliformis.</i>						
After 12 days	+++	+++	*+++	*+++	+++	+++
After 30 days	+++	+++	*+++	*+++	+++	+++
<i>B. Pyocyaneus.</i>						
After 12 days	++	+++	*+	*++	+	++
After 30 days	+++	+++	*+++	*+++	+++	+++

* means that the agar slopes were inoculated immediately after taking the meat broth tubes from the anaerobic tin.

Table 1.

	Anaerobic: continual.		Anaerobic: intermittent.		Aerobic: continual.	
	+R.B.	-R.B.	+R.B.	-R.B.	+R.B.	-R.B.
B. typhosus 3 days.	2960	5220	2960	5160	3320	4016
5 days	600?	5136	2320	4600	1600	4800
7 days	640	1000	960	1160	1400	3680
9 days	Tube broken	24	240	2988	408	520
11 days	-	104	336	620	768	960
13 days	-	1080	320	1160	760	960
15 days	-	0	0	14	412	87
17 days	-	1552	1184	1004	1552	1280
Average.	1400	1764	1040	1963	1277	2038
Stained Films at end of culture.	—	R.B. absent B. Typhosus plentiful.	Reading bacillus & Spores in quantity. Very few B. Typhosus.	R.B. ab- sent. B. Typhosus plenti- ful.	Reading bacillus & spores in quan- tity. Very few B. Typh- osus.	R.B. absent. B. Typh- osus plenti- ful.

Table 2.

	Anaerobic: continual.		Anaerobic: intermittent.		Aerobic: continual.	
	+R.B.	-R.B.	+R.B.	-R.B.	+R.B.	-R.B.
B. Paratyphosus B. 3 Days.	2040	7200	2800	1920	2456	3552
5 Days.	5560	104	5420	735	1520	960
7 Days.	0	0	100	1396	680	896
9 Days.	2090	Innumerable	Innumerable	1680	Innumerable	868
11 Days.	1750	1600	2070	1544	1448	1400
13 Days	Innumerable	1000	2056	1240	960	2240
15 Days	96	72	144	Innumerable	1320	Innumerable.
17 Days	120	Innumerable	2160	1056	1090	1416
Averages	1665	1663	2107	1369	1353	1619
Stained films at end of culture.	R.B. in quantity B. Para B plentiful.	R.B. absent. B. Para B. plentiful	R.B. mostly in spore-form. B. Para B plentiful.	R.B. absent. B. Para B. plentiful.	R.B. in quantity. B. Para B plentiful.	R.B. absent. B. Para B. plentiful.

Table 3.

	Anaerobic: continual.		Anaerobic: intermittent.		Aerobic: continual.	
	+ R.B.	- R.B.	+ R.B.	- R.B.	+ R.B.	- R.B.
B. dysenteriae Shiga. 3 Days.	2120	330	2416	160	0	3280
5 Days	1408	2040	2040	2560	1696	1970
7 Days	2690	0	2712	12	1725	36
9 Days	Innumerable	Innumerable	2240	1200	1600	1440
11 Days	3200	270	2	720	1905	1520
13 Days	400	456	104	288	800	480
15 Days	Innumerable	880	2120	960	600	640
17 Days	248	240	2400	656	1280	600
Averages	1677	602	1754	819	1200	1246
Stained films at end of culture.	R.B. in quantity. B. Shiga in small numbers.	R.B. absent. B. Shiga plentiful.	R.B. in quantity B. Shiga in small numbers	Gram-positive Contamination. B. Shiga in small numbers.	R.B. chiefly in spore form. B. Shiga in small numbers.	R.B. absent. B. Shiga in small numbers.

Table 4.

	Anaerobic: continual.		Anaerobic: intermittent.		Aerobic: continual.	
	+ R.B.	- R.B.	+ R.B.	- R.B.	+ R.B.	- R.B.
B. dysenteriae Flexner. 3 Days.	4920	2680	6200	4480	5944	3120
5 Days	1930	2296	2152	2800	2800	2520
7 Days	280	448	208	360	744	2240
9 Days	288	600	740	1212	1456	1200
11 Days	390	424	260	560	810	968
13 Days	856	424	2152	1680	536	1240
15 Days	0	92	208	132	156	698
17 Days	1360	1160	2160	1440	1472	1520
Averages	1253	1015	1760	1583	1739	1698
Stained films at end of culture.	R.B. in quantity chiefly as spores. B. Flexner plentiful	R.B. ab- sent. B. Flex- ner plenti- ful.	R.B. in quantity chiefly as spores. B. Flexner plentiful	R.B. absent B. Flexner plentiful.	R.B. mainly as spores. B. Flexner plentiful	R.B. absent. B. Flexner plentiful.

These results are not very convincing one way or the other, but so far as they go, they seem to indicate that, with the exception of *b.typhosus*, no pronounced inhibitory effect is produced by the growth in symbiosis of the Reading bacillus. Even in the case of the possible exception, the difference is not sufficiently well marked to enable one to say that the Reading bacillus has had any effect on the *b.typhosus*. These findings are again partially corroborated by the work previously done in comparing the flora of wounds before and after treatment with salt-bags. Pathogenic organisms do not appear to be crowded out or inhibited as to their growth by the presence of the Reading bacillus. It does not appear, therefore, that the beneficial action of the latter organism is to be explained in this way.

At the same time, the result of film examinations seems to indicate that it might be worth while to add to a series of broths of given amount, definitely ascertained numbers of the different organisms and, after incubation for various periods of time, to make counts of the relative numbers present, by plate culture, to determine whether the two types of organism present in each tube multiply at the same rate or whether one increases out of proportion to the other. Whether the Reading bacillus increases or not has not been/

been determined in the foregoing experiments and the films seem to suggest a possible interaction inasmuch as in some the Reading bacillus is present in apparently small numbers and frequently mainly in the spore form, whereas in others it is present in large numbers in its typically active form. At the same time, the foregoing results do not appear to be very encouraging and do not seem to indicate that the beneficial action will be explained along these lines.

B. Experimental Inoculation with Pathogenic Anaerobes grown in Symbiosis with the Reading Bacillus.

Having been unable to produce any pathogenic effects on animals by the methods described, I determined to find out if, by growing the Reading bacillus in symbiosis with certain pathogenic organisms, the lethal action of the latter could be counteracted. The two pathogenic organisms chosen for this purpose were vibron septique strain "Amalzi" and bacillus perfringens, for both of which I am indebted to Miss Muriel Robertson. Each of these was grown in symbiosis with the Reading bacillus on various media, while pure cultures of each of the former were used as controls.

The results of animal inoculation with these various combinations are exposed in the following tables. Unfortunately the only animals available were rabbits, many of which kept dying off before they could be used for inoculation purposes. They were not the most suitable animals for the purpose but the best that I was able to obtain.

Table 1. Combinations with B. Perfringens.

<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>	<u>Result.</u>
1. 780 grams.	3 cc. of meat broth culture of R.B. and B.P. grown together for 3 days.	Thigh muscles.	Death in 24 hours. Fluid oedema, gas & haemorrhages in muscles of inoculated thigh.

<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>	<u>Result.</u>
2. 990 grams.	3 cc of dialysed meat broth culture of R.B. & B.P. grown together for 7 days.	Ditto.	Death after 14 days due to heavy infection with coccidial cysts. No local lesions.
3. 430 grams.	2 cc. of meat broth culture of B.P. & R.B. grown together for 7 days.	Ditto.	Death after 27 days from coccidiosis.
4. 550 grams (control)	1 cc. of meat broth culture of B.P. only, grown for three days.		Death in 48 hours with great swelling and oedema of tissues.
5. 770 grams (control)	1 cc. of meat broth culture of B.P. grown for 9 days.	Thigh muscles	Death after 48 days from coccidiosis. Great emaciation.
6. 470 grams (control)	3 cc. of dialysed meat broth culture of B.P. grown for 7 days.	Ditto.	Death after 27 days due to coccidiosis. No local lesions.
7. 1140 grams (control)	2 cc. of suspension in broth from 2 agar slope cultures of B.P. grown for three days.	Ditto.	Death in 16 days due to coccidiosis. No local lesions.
8. 930 grams	3 cc. being a mixture in equal proportions from agar slope cultures of B.P. and of R.B. grown separately for 3 days.	Ditto.	Slight swelling after injection which disappeared. Death in 27 days from coccidiosis.

	<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>	<u>Result.</u>
9.	385 grams	0.5 cc. of broth culture of B.P. & R.B. grown together.	Ditto.	Death in 24 hours. No local lesion of any sort.
10.	820 grams	3 cc. being a mixture in equal parts of a suspension in broth of R.B. & of a broth culture of B.P. grown for 3 days.	Ditto.	Death after 17 days from coccidiosis. No local lesions.

Table 2. Combinations with Vibrion Septique.

1.	1590 grams	4 cc. of dialysed meat broth culture of V.S. & R.B. grown together for 7 days.	Thigh muscles	Quite well 27 days afterwards.
2.	470 grams	2 cc. of meat broth culture of V.S. & R.B. grown together for 10 days.	Ditto.	Death in 24 hours. Oedema and gas.
3.	310 grams	2.5 cc. of meat broth culture of V.S. & R.B. grown together at 37° cent. for 4 days and then for 49 days at room temperature.	Ditto.	Death in 18 hours. Fluid oedema, gas & haemorrhages.
4.	550 grams	3 cc. being equal parts of meat broth culture of R.B. & of V.S. grown separately for 3 days.	Ditto.	Death in 48 hours. Slight swelling of thigh muscles only. No gas and no discoloration.

<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>	<u>Result.</u>
5. 540 grams	3 cc.made up of equal parts of a broth culture of V.S. and of a meat broth culture of R.B. grown for 3 days.	Ditto.	Death in 19 hours with marked fluid oedema, gas & haemorrhages.
6. 2000 grams (control)	5 cc.of dialysed meat broth culture of V.S. grown for 7 days.	Ditto.	Death in 24 hours with marked fluid oedema, gas & haemorrhages.
7. 2000 grams (control)	2 cc.of meat broth culture of V.S. grown for 7 days.	Ditto.	Ditto.
8. 750 grams (control)	1.5 cc.of meat broth culture of V.S.grown for 10 days.	Thigh muscles	Death in 17 hours with fluid oedema, gas and haemorrhages.
9. 510 grams	0.5 cc.of broth culture of V.S. & R.B.grown together.	Ditto.	Death in 24 hours. No local lesion of any sort.
10. 530 grams	1 cc.of broth culture of V.S. & R.B. grown together.	Ditto.	Death in 25 days from coccidiosis. No local lesion.
11. 500 grams	3 cc.of broth culture of V.S. & R.B. grown for 10 days.	Ditto.	Death in 17 hours with intense fluid oedema, gas & haemorrhages.
12. 1050 grams	4 cc.being equal parts of broth culture of V.S. & of R.B. grown separately for 10 days.	Ditto.	Death after 9 days from coccidiosis. No local lesions.

	<u>Weight.</u>	<u>Material.</u>	<u>Route.</u>	<u>Result.</u>
13.	590 grams (control)	1 cc. of broth culture of V.S.	Thigh. muscles.	Death in 24 hours with fluid oedema, gas & haemor- rhages.
14.	720 grams (control)	2 cc. of broth culture of V.S. grown for 3 days.	Ditto.	Death in 24 hours with fluid oedema, gas & haemor- rhages.
15.	510 grams (control.)	1.5 cc. of broth culture of V.S. grown for 10 days.	Ditto.	Death in 24 hours with fluid oedema, gas & haemor- rhages.
16.	340 grams (control)	2 cc. of broth culture of V.S. grown for 4 days at 37° cent and for 49 days at room tempera- ture.	Ditto.	Death in 24 hours with fluid oedema, gas & haemor- rhages.

To take the combinations of Reading bacillus and bacillus perfringens first, the experiments above enumerated do not appear to show, so far as they go, any beneficial effect from the presence of the former bacillus. They seem to indicate that perfringens cultures, whether alone or in symbiosis with the Reading bacillus, are capable of killing within a few hours, if the culture be only 2 or 3 days old. Cultures of perfringens with or without the Reading bacillus, appear to have lost their toxic effect after 7 days incubation, so that the presence of the Reading bacillus/

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bacillus in such has nothing to do with the prevention of a fatal issue. Whether the organisms are grown in dialysed or in non-dialysed meat broth does not appear to make any material difference.

Further, simple agar suspensions of bacillus perfringens are devoid of any ill effects and the presence of the Reading bacillus in such cases can have no value.

There are two doubtful experiments in the first series. In one case the animal died in 24 hours after the injection of 0.5 cc. of a symbiotic culture. Unfortunately, the age of the culture has not been recorded but as there was no local lesion, it is doubtful if the death can be attributed to the inoculation. In the other case, while 1.5 cc. of a 3 day broth culture of vibron septique was inoculated along with 1.5 cc. of a broth suspension from an agar slope of the Reading bacillus no ill effect was observed. It may mean that in such a combination, the animal is protected from the effects of the bacillus perfringens but the evidence is too scanty to allow of any deduction being drawn.

In the second series of experiments, the evidence that the Reading bacillus plays any important part in preventing a fatal issue when introduced along with the/

the vibron septique is again inconclusive. In the case of the latter organism, the age of the culture does not appear to matter. Cultures of the vibron septique 53 days old appeared to be as lethal as cultures only two or three days old.

Where the organisms are grown symbiotically in meat broth, no protection is afforded. Where dialysed meat broth is used, however, there is some indication that the presence of the Reading bacillus does exert a beneficial effect. No.1 animal, although given a fairly large dose of the symbiotic mixture, suffered no ill effects, while a control animal (No.6), although given a slightly smaller dose per body weight of a dialysed culture of vibron septique alone, grown for the same length of time, was dead in 24 hours. It is possible, therefore, that a further investigation of dialysed cultures may throw some light on the action of the Reading bacillus.

Cultures in ordinary broth of vibron septique appear all to be rapidly fatal. Where, however, the Reading bacillus has been grown in the broth symbiotically with vibron septique, the inoculation of the resulting growth does not appear to be invariably followed by death. Four animals were so inoculated. One died for no apparent reason and must be left out of account. One died with typical symptoms, while the/

the other two survived. One of the latter was inoculated with broth cultures of vibriion septique and of Reading bacillus grown separately. Whether the survival of the animal was due merely to accident or whether the dose per body weight had anything to do with the question requires further investigation.

The main point to recognise is that the evidence of any protective action on the part of the Reading bacillus is, from the point of view of animal experiment, so far extremely inconclusive. There are indications that, by following up certain lines of inquiry suggested by these experiments, some fresh light may be shed on the question. At the same time, it must be borne in mind that the conditions of experiment are vastly different from those which obtain in open wounds, such as are met with here. In the case of animals, not only is the material injected into what are closed tissues but the material consists not only of the living bacteria but also of their products of decomposition. Those which are formed by the activity of the pathogenic member are highly toxic and unless these toxins have been split up into non-toxic elements by the action of the Reading bacillus, the animal will suffer much the same way as if only a pure culture of the pathogenic organism had been injected./

injected.

It seems to me that the Reading bacillus may exert its beneficial action, where anaerobes capable of producing toxic effects are present, in one of several ways.

It may, by more rapid growth, use up the only pabulum it can grow upon, viz., the dead tissue, and so minimise or prevent the activities of the pathogenic organisms. In other words, its chief action may be due to its proteolytic activity in removing the dead tissue, which forms the base from which pathogenic organisms deliver their attacks. This possible explanation has already been referred to and was the first conception which I formed of the way in which it brought about its beneficial results. It may be due to the fact that in the presence of the Reading bacillus, the growth of the pathogenic anaerobes is inhibited and that while they may still be present, they are unable to form sufficient toxic products to damage the patient, while in the meantime, the Reading bacillus is rapidly removing the tissue from which alone these pathogenic anaerobes can form their toxic products. Some light may be thrown on the possible explanation by growing a pathogenic anaerobe in symbiosis with the Reading bacillus, the culture tube being inoculated with known numbers of each. At intervals, /

intervals, measured samples of the cultures might be plated out and the relative increase in numbers of the two kinds of organism present noted. This experiment I mean to carry out in the near future. It ought to show whether the Reading bacillus is able to flourish while the pathogenic member remains stationary in point of numbers.

It may be, on the other hand, that, apart from its proteolytic action on the dead tissues present in a wound, the same action is at work on the toxins or toxic products formed as a result of the pathogenic organism's activity. That some such explanation is probable seems indicated by the rapid improvement in the patient's general condition that usually sets in on the third or fourth day after a wound has been sown with the Reading bacillus. As I have already said, it seems difficult to believe that it is merely due to its proteolytic action on the dead tissue only. Whatever be the nature of these toxins or toxic products, it is possible that the Reading bacillus may either split them up into non-toxic elements or so alter the tissue from which they are derived as to prevent the formation of such by the pathogenic members present. This problem which will entail a good deal more work, I have commenced to investigate and the results so far obtained are shown in the next section.

C. Experimental Inoculation with Toxic Filtrates in which Reading Bacillus had been grown.

In order to test the hypothesis, suggested by a consideration of the foregoing experiments, viz., that the Reading bacillus might have a beneficial action by splitting up toxins or toxic products as well as the proteins of dead tissues, I carried out three experiments with tetanus toxin. The latter was kindly placed at my disposal by Dr A. T. MacConkey, superintendent of the Serological Department of the Lister Institute, Elstree. Unfortunately, in choosing tetanus toxin, I found myself confronted by a difficulty that I had not taken into account, viz., the instability of the toxin which deteriorates rapidly on manipulation and on incubation at 37° cent. The minimal lethal dose as determined by MacConkey was no longer reliable by the time I was ready to use it for inoculation. Owing to the scarcity of animals I was unable to determine the M.L.D. for myself and the first series of inoculations were therefore made in order to find out roughly a dose that would kill within a fairly short time. These do not concern the main question here and are therefore omitted. Having in this way learned what doses to use, the toxin/

toxin was tubed out into a series of culture tubes. One of these was sown with the Reading bacillus, one with vibriion septique, one with b.perfringens, one with b.typhosus and one was left uninoculated. I desired to know whether, if the growth of the Reading bacillus detoxicated the filtrate, other anaerobes by their growth in the tetanus toxin would do the same. All were incubated anaerobically at 37° Cent. for 4 days and then at room temperature for 4 days.

Guinea pigs were used and the material inoculated into the thigh muscles. The results are tabulated as follows:-

<u>Amnt. of Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
1. 0.42 cc.	Tet. Toxin and b.Typhosus.	Dead in 4 days after showing typ- ical tetanus.
2. 0.4 cc.	Tet. Toxin and V.Septique.	Dying in 4 days with typical tetanus.
3. 0.59 cc.	Tet. Toxin and B.Perfringens.	Dead in 2 days apparently of some infection. No evidence of gas or haemorrhages.
4. 0.6 cc.	Tet. Toxin alone.	Dead in 3 days after showing typical tetanus.
5. 0.9 cc.	Tet. Toxin & Reading Bacil- lus.	Dead in 7 days but only began to show spasms on the 4th day.

A second supply of tetanus toxin was procured and tubed out as before. Three were inoculated with the Reading bacillus, one with *b. perfringens*, one with *vibrion septique* and three were left uninoculated. All were grown anaerobically at 37° Cent. for 4 days. Growth had occurred in all those inoculated with the exception of that having *vibrion septique*. The growth in this tube, if any, was scanty. Guinea pigs were inoculated as before and the results are noted in the next table:-

Table 2.

Amount of Tet. Tox.
pro 100 grams of
body weight.

Material.

Result.

1. 0.235 cc.	Tet. Toxin alone.	Stiffness appeared in 44 hours. Death in 4½ days with typical tetanus.
2. 0.25 cc.	Tet. Tox. and R.B.	Fnd. dead on 6th day without having mani- fested any sign of tetanus and with no local lesion.
3. 0.25 cc.	Tet. Toxin alone.	Marked spasm in 44 hours. Dead on 5th day of general tetanus.
4. 0.529 cc.	Tet. Toxin and R.B.	Fnd. dead on 6th day, the only sign of tetanus being a slight stiffness in one leg noticed for the first time the previous day. He could use the leg quite freely however.

5.	0.53 cc.	Tet. Toxin & ? Vib. Sept.	Marked spasm in 44 hours. Dead on 5th day after having developed generalised tetanus.
6.	0.57 cc.	Tet. Toxin & B. Perfringens.	End. dead within 24 hours without having developed tetanus, but with a haemorrhagic condition of thigh muscles - probably result of bacillary action.
7.	0.71 cc.	Tet. Toxin only.	Spasm noticed within 44 hours. Dead on 5th day after having developed generalised tetanus.
8.	1.1 cc.	Tet. Toxin & R.B.	End. dead on 5th day, able to use limbs quite well with just a slight stiffness in the muscles of the inoculated thigh. No typical tetanus. No local lesion.

So far as these experiments go, they show that tetanus toxin in which the Reading bacillus has been grown, has been profoundly altered in some way by the growth of that organism. Out of the first series, the animal inoculated with tetanus toxin in which the Reading bacillus had been grown, lived longer than any of the others, although given a considerably larger dose and only began to show spasms after 4 days, /

days, by which time all the other animals were dead. The control animal inoculated with only two-thirds this dose of tetanus toxin in which, however, nothing had been grown, died on the third day of generalised tetanus. The presence of *b. typhosus* and of *vibrio septique* had no obvious effect on the tetanus toxin in which they were grown, and these animals died from tetanus. The animal inoculated with tetanus toxin in which *perfringens* had been grown, may have died from a *perfringens* infection, but there was no typical evidence of such.

The results detailed in the second table corroborate further what was found above. The smallest dose of tetanus toxin by itself which was injected with fatal results was 0.235 cc. per 100 grams of body weight. Spasms began in 44 hours and the animal was dead in $4\frac{1}{2}$ days. One of the animals inoculated with more than double this dose of toxin in which, however, the Reading bacillus had been grown, lived for 6 days. It never manifested any visible sign of tetanus beyond a slight stiffness in the inoculated limb which, however, did not prevent the animal using it freely for locomotion. Another which had slightly more than four times the smallest dose of tetanus toxin which by itself was found to cause spasms and death in $4\frac{1}{2}$ days, /

days, lived till the fifth day. Here again, beyond a very slight stiffness, almost imperceptible, the animal showed no sign of tetanus.

The net result of the above series of experiments amounted to this, namely, that the Reading bacillus alone of all those tested was able in some way to modify very considerably the toxin of tetanus. The experiments, however, were inconclusive, but the results were sufficiently encouraging to lead me to make farther investigations in a more accurate manner along similar lines.

Fluid tetanus toxin, for the reasons I have already mentioned, was unsuitable, and it became necessary to get a more stable preparation. This I was enabled to obtain through the kindness of Dr. R. A. O'Brien, Director of the Wellcome Physiological Research Laboratories, who supplied me with a small quantity of dried tetanus toxin. Its degree of toxicity had been determined by Dr. O'Brien, who had also satisfied himself that it would remain stable for two or three weeks at least after solution. Being thus in possession of a toxin of known stability, and whose minimal lethal dose had previously been ascertained, I had a fixed point from which to start/

start comparative experiments. The latter were spread over several months, according as I was able to obtain supplies of animals. Guinea-pigs were used exclusively throughout the following experiments. The technique was as follows. A known quantity of the toxin was accurately weighed out, observing due precaution to prevent error arising from absorption of moisture by the hygroscopic powder. This amount was then dissolved in a previously sterilised solution of 0.85% sodium chloride in water.

From this, measured amounts were pipetted off and delivered into known volumes of sterile nutrient broth, in order to obtain dilutions of tetanus toxin of known strength. Some of these were then inoculated with the living organism whose effect on the toxin I wished to observe, while certain others were left uninoculated to serve as controls. The whole of the tubes, inoculated and uninoculated, in any one experiment, were then incubated anaerobically in the same apparatus at 37° Cent. for the same length of time. At the end of incubation, the inoculated broths were as a rule filtered through candles to remove the living organisms. Although sterile, the contents of the control tubes containing only tetanus toxin in broth were likewise filtered so that they might be subjected/

subjected to the same manipulation as the inoculated tubes. In the later experiments, however, this was not always done in the case of the control tubes, as it was found that the filtering in no way affected the M.L.D. of the toxin.

Further dilutions were then made where necessary, using sterile sodium chloride solution (0.85%) so as to obtain the necessary range of doses required for inoculation. Each animal was carefully weighed and the required dose inoculated into the muscles of one thigh. In the case of each animal the time was noted when signs of tetanus were first observed, and the date of death recorded when a fatal issue ensued. Having satisfied myself in a preliminary experiment not recorded here that the dessicated tetanus toxin would act as I had been led to believe it would, various series of experiments were carried out at different times and the data are recorded in the following pages.

The first series of experiments were undertaken to determine, by the inoculation of certain graduated doses, the effect on O'Brien's Tetanus Toxin of various organisms grown under the conditions above described. The organisms employed for this purpose were/

were *b. perfringens*, *vibrio septique*, *b. histolyticus*, *b. sporogenes* (Metchnikoff)* and the Reading bacillus. Perhaps it may be opportune to state here that the dessicated tetanus toxin was stated by Dr. O'Brien to be able to kill a 300 gram guinea-pig in a dose of 0.000004 of a gram, i.e. 0.0000013 gram for every 100 grams of body weight. To facilitate comparison I have expressed the doses in the following experiments in the latter terms, viz. as so much of a gram for every 100 grams of body weight.

1.FIRST SERIES OF EXPERIMENTS./

I. FIRST SERIES OF EXPERIMENTS.

1. Control animals inoculated with broth containing Tetanus Toxin only, incubated anaerobically at 37° Cent. for 6 days.

	<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
I.	0.00000069 grams	Filtrate from broth contain- ing Tetanus Toxin only.	First sign of tetanus observed in 62 hours. Died of tetanus in 24 days.
II.	0.00000087 grams	Ditto.	First sign of tetanus observed in 62 hours. Died of tetanus in 11 days.
III.	0.0000009 grams	Ditto.	First sign of tetanus observed in 36 hours. Died of tetanus in 12 days.
IV.	0.000002 grams	Ditto.	First sign of tetanus observed in 36 hours. Died of tetanus in 6 days.
V.	0.000003 grams	Ditto.	First sign of tetanus observed in 36 hours. Died of tetanus in 4 days.

2. Animals inoculated with broth containing Tetanus Toxin exposed to the action of b. perfringens for 6 days anaerobically.

	<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
I.	0.00000085 grams	Filtrate from culture of b. Perfringens in broth containing Tet. Toxin.	Rigidity of some of thigh muscles in 36 hours. Died of tetanus in 36 days.
II.	0.000002 grams	Ditto.	Rigidity of some of thigh muscles in 36 hours. Died of tetanus in 10 days.
III.	0.000003 grams	Ditto.	First signs of tetanus noticed in 36 hours. Died of tetanus in 10 days.

3. Animals inoculated with broth containing Tetanus Toxin acted on by Vibrion septique for 6 days anaerobically.

I.	0.000001 grams	Filtrate from culture of Vibrion septique grown in broth containing Tet. Toxin.	First sign of tetanus observed in 36 hours. Died of tetanus in 8 days.
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Amount of Tetanus
Toxin pro 100
grams of body
weight.

Material.

Result.

II.	0.000002 grams.	Filtrate from culture of Vibriion septicque grown in broth containing Tet. Toxin.	First sign of tetanus observed in 36 hours. Died of tetanus in 4 days.
III.	0.000003 grams	Ditto	First sign of tetanus observed in 36 hours. Died of tetanus within 4 days.

4. Animals inoculated with broth containing Tetanus
Toxin which had been exposed for 6 days
anaerobically to the action of b. histolyticus.

I.	0.000001 gram.	Filtrate from culture of b. Histolyticus grown in broth containing Tet. Toxin	First sign of tetanus observed in 62 hours. Died of tetanus in 11 days.
II.	0.000002 grams	Ditto	First sign of tetanus observed in 36 hours. Died of tetanus in 10 days.
III.	0.000003 grams	Ditto	First sign of tetanus observed in 36 hours. Died of tetanus in 3 days.

5. Animals inoculated with broth containing Tetanus Toxin which had been exposed for 6 days anaerobically to the action of b. Sporogenes (Metchnikoff).

	<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
I.	0.00000098 grams	Filtrate from culture of b. Sporogenes in broth containing Tet. Toxin.	No outward sign of tetanus whatever. Died at end of 39 days.
II.	0.00000019 grams	Ditto	Dead in 32 days. No visible sign of tetanus.
III.	0.00000028 grams	Ditto	Dead in 36 days. No visible sign of tetanus.
IV.	0.00000043 grams	Ditto	Dead in 47 days. No visible sign of tetanus.

6. Animals inoculated with broth containing Tetanus Toxin which had been exposed for 6 days anaerobically to the action of the Reading bacillus.

I.	0.00000004 grams	Filtrate from culture of Reading bacillus grown in broth containing Tet. Toxin	Never showed signs of Tetanus. Died 42 days later.
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Amount of Tetanus
Toxin pro 100
grams of body
weight.

Material.

Result.

II.	0.00000083 grams	Filtrate from culture of Reading bacillus grown in broth containing Tet.Toxin	Never showed signs of Tetanus. Remained alive and well.
III.	0.00000092 grams	Ditto	Never developed Tetanus. Remained alive and well.
IV.	0.0000018 grams	Ditto	Never developed Tetanus. Found dead 23 days later.
V.	0.0000027 grams	Ditto	Never developed Tetanus. Found dead in 42 days.
VI.	0.0000036 grams	Ditto	Never developed Tetanus. Remained alive and well.
VII.	0.000005 grams	Ditto	Never developed Tetanus. Remained alive and well.

From the foregoing series of experiment we learn that tetanus toxin not exposed (at) organismal action but treated otherwise as in the infected tubes was able to produce signs of tetanus even in the smallest dose employed, viz. .00000069 gram. pro 100 grams of body weight. As the M.L.D. was stated to be .00000013 gram pro 100 grams of body weight the above dose followed by tetanic signs is almost equivalent to half the reputed M.L.D. The toxic effects became manifest in sixty-two hours, and the animal was dead in twenty-four days. The remaining five guinea-pigs which received increasing doses of the toxin all developed tetanus and died; those which received larger amounts succumbing more quickly.

The 3 animals inoculated with tetanus toxin which had been exposed to the vital activity of *b. perfringens* all developed tetanus of which they died. The smallest dose employed here was rather less than the asserted M.L.D. of pure toxin, but the symptoms began in 36 hours. While these animals eventually died of tetanus they lived rather longer than the guinea-pigs inoculated with similar amounts of pure toxin. Similarly all the animals inoculated with tetanus toxin which had been exposed to the action/

action of *Vibrio septique* died of tetanus. The smallest dose injected was very slightly less than the reputed M.L.D. but tetanic signs appeared within 36 hours and the animal was dead in eight days, corresponding very closely to the time taken by the pure tetanus toxin to kill.

All the animals inoculated with tetanus toxin in which *b. histolyticus* had been grown, developed tetanus and died. The smallest dose given was again rather less than the reputed M.L.D. and the animal died in eleven days, again corresponding closely to the time required by the pure toxin to kill.

In the case of tetanus toxin in which *b. Sporogenes* was grown no sign of tetanus was noticed during the life of any of the four animals inoculated although the largest dose given was about three and a half times the reputed M.L.D. and about seven and a half times the lowest dose found in the control experiment to kill. They all died, however, for what reason I cannot state definitely, but it is perhaps worthy of note that the animal which received the highest dose of the toxin acted upon by *sporogenes* lived longer than the animal which had the smallest dose.

Lastly/

Lastly we come to tetanus toxin exposed to the action of the Reading bacillus. The animal which received the highest dose, nearly four times the reputed M.L.D. and seven times the lowest dose pure tetanus toxin found capable of killing in the control experiments, never developed tetanus, and was alive and well months later. Altogether in this set seven animals were inoculated with varying doses. None ever showed any sign of tetanus, although three died, two after forty-two days and one after twenty-three days. One of the fatal cases had received much less than the reputed M.L.D. so that in these 3 cases it is doubtful if the death of the animals had any relationship to the tetanus toxin injected. It was evident from the above that the Reading bacillus and b. Sporogenes alone of all those investigated in the above experiments, were able in some way to modify tetanus toxin so completely that no sign of tetanus manifested itself in the inoculated animals.

To determine in the next place how large a dose of tetanus toxin previously exposed to the action of the Reading bacillus could be borne by the animal without manifesting signs of tetanus, another series of experiments was undertaken. The procedure/

procedure

was much the same as before. In this case, however, nutrient broth of a+5 reaction was substituted for broth of the ordinary+10 reaction. This change was made since from my previous work it was known that broth having the former reaction formed a better culture medium for the Reading bacillus than broth of the usual reaction. At the same time a parallel series of tubes was put up in which precipitated chalk had been added to+10 nutrient broth with the idea of neutralising acids which might be formed in the course of the specific anaerobe's growth. Controls were also prepared in an exactly comparable way, excepting that no organisms were added.

In view of the very strong proteolytic properties possessed by *b. histolyticus*, I decided to test this organism once more, using however, on this occasion +5 broth.

II. SECOND SERIES of EXPERIMENTS.

1. Control animals inoculated with varying doses of Tetanus toxin contained in +5 broth which had been incubated anaerobically for 7 days but unacted upon by any organisms.
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<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>		<u>Material.</u>	<u>Result.</u>
I.	0.000001 gram.	Filtrate from +5 broth con- taining Tet- anus Toxin only	First sign of tetanus observed in 24 hours. Died of tetanus in 6 days.
II.	0.000003 grams	Ditto	First sign of tetanus observed in 24 hours. Died of tetanus in 3 days.
III.	0.0000046 grams	Ditto	First sign of t. tetanus observed in 24 hours. Died of tetanus in 4 days.

2. Control animals inoculated with varying doses of Tetanus Toxin in +10 broth containing chalk incubated as above but unexposed to organismal interference.
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<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>		<u>Material.</u>	<u>Result.</u>
I.	0.000001 gram	Filtrate from +10 broth containing 20/o chalk and Tetanus Toxin	First sign of tetanus observed in 17 hours. Died of tetanus in 3 days.
II.	0.0000029 grams	Ditto	First sign of tetanus observed in 17 hours. Died of tetanus in 3 days.
III.	0.0000046 grams	Ditto	First sign of tetanus observed in 17 hours. Found dying of tetanus and killed at the end of 36 hours.

3. Animals inoculated with varying doses of Tetanus Toxin in +5 broth in which the Reading bacillus had been grown under the conditions above stated.

I.	0.000001 gram.	Filtrate from culture of Reading bacillus grown in +5 broth containing Tet. Toxin.	Never any sign of tetanus. Remained alive and well.
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	<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
II.	0.000003 grams	Filtrate from culture of Reading bacillus grown in +5 ^{broth} containing Tet. Toxin.	Never any sign of tetanus. Remained alive and well.
III.	0.0000047 grams	Ditto	Ditto
IV.	0.0000073 grams	Ditto	Ditto
V.	0.0000091 grams	Ditto	Ditto
VI.	0.000021 grams	Ditto	Ditto
VII.	0.00002 grams	Ditto	Ditto
VIII.	0.000061 grams	Ditto	Ditto
IX.	0.000078 grams	Ditto	Ditto
X.	0.00008 grams	Ditto	Showed very slight stiffness in inoculated limb on 7th day, but recovered in 2 days' time and has remained well ever since.

4. Animals inoculated with varying doses of Tetanus Toxin in +10 broth to which chalk had been added and in which the Reading Bacillus had been grown under the conditions above stated.

I.	0.000001 gram..	Filtrate from culture of Reading bacillus grown in +10 broth containing Tet. Toxin and 2 ^o /o chalk	Never showed any sign of Tetanus. Remained alive and well.
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Amount of Tetanus
Toxin pro 100
grams of body
weight.

Material.

Result.

II.	0.000003 grams	Filtrate from culture of Reading bacillus grown in +10 broth containing 20% chalk.	Never showed any sign of tetanus. Remained alive and well.
III.	0.0000046 grams	Ditto	Ditto
IV.	0.0000073 grams	Ditto	Ditto
V.	0.000009 grams	Ditto	Showed very slight stiffness in inoculated limb on 7th day, but had entirely recovered in 2 days' time and has remained well.
VI.	0.000019 grams	Ditto	Showed very slight stiffness in inoculated limb on 6th day, but had entirely recovered in 3 days' time. Animal remained alive and well.
VII.	0.000021 grams	Ditto	Showed some stiffness in inoculated limb on the 7th day, but this had all disappeared in 6 days. Animal remained alive and well.
VIII.	0.00006 grams	Ditto	Showed very slight stiffness in inoculated limb on 6th day, but had entirely recovered in 3 days' time. Animal remained alive and well.

	<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
IX.	0.00008 grams	Filtrate from culture of Reading bacillus grown in +10 broth containing 20/o chalk.	Showed some stiffness of inoculated limb on 6th day, and this persisted for 7 days. Animal recovered and remained alive and well.
X.	0.000096 grams	Ditto	Shewed complete rigidity of inoculated limb on the 6th day. The animal gradually recovered use of limb in 12 days and remained alive and well.
XI.	0.000148 grams	Ditto	Showed complete rigidity of inoculated limb on 6th day. Limb remained stiff for some time, but gradually recovered in 3-4 weeks' time, and has since remained alive and well.

5. Animals inoculated with varying doses of Tetanus

Toxin in +5 broth in which b. histolyticus
had grown under the conditions above stated.

I.	0.000001 gram.	Filtrate from culture of b. Histolyticus grown in +5 broth containing Tetanus Toxin.	First sign of Tetanus observed in 24 hours. Died of tetanus in 84 hours.
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Amount of Tetanus

Toxin pro 100
grams of body
weight.

Material.Result.

II.	0.0000096 grams	Filtrate from culture of b. Histolyticus grown in +5 broth containing Tetanus Toxin.	First sign of tetanus observed in 17 hours. Died of tetanus in 4 days.
III.	0.000025 grams	Ditto	First sign of tetanus observed in 17 hours. Died of tetanus in 60 hours.

In this series the lowest dose of tetanus toxin alone in + 5 broth was very slightly under the reputed M.L.D. Signs of tetanus were evident within 24 hours and the animal died of generalised tetanus in six days.

In the case of the second set, the lowest dose employed was exactly as in the first set, but instead of being contained in +5 broth, it had been incubated in +10 broth containing chalk. As in the first set no organisms had been added. The animal receiving this dose showed signs of tetanus in seventeen hours and was dead in three days.

All the animals inoculated with uninfected tetanus toxin died of generalised tetanus, but those in the second set, in which the tetanus toxin had been/

been incubated in +10 broth containing chalk, showed signs of tetanus and died sooner than the animals in the first series. Why this should be so does not concern this thesis, although it is worthy of note in passing.

In the third set of Experimental Animals, viz., those inoculated with tetanus toxin contained in +5 broth infected with the Reading bacillus, none of them died. Further, with the exception of one, (that which had the highest dose,) none of the others showed the slightest sign of tetanus. The animal which had nearly sixty-two times the lowest dose given in the control series showed slight stiffness on the seventh day, but had quite recovered in two days and was alive and well months afterwards.

In the fourth set of animals inoculated with tetanus toxin in +10 broth containing chalk, the whole having been exposed to the action of the Reading bacillus, a rather greater range of dosage was employed. Not one of the eleven inoculated animals died, although the highest dose given was about 114 times the reputed M.L.D. and about 148 times the lowest dose of the parallel control series which proved fatal in three days. There was one point/

point of difference between the third and the fourth sets of experimental animals, viz. that a larger number of the latter showed signs of local tetanus. Six out of eleven of the fourth set were effected in varying degrees, mostly slightly, as compared with 1 out of 10 in the series where the organism had been grown in + 5 broth.

I have already mentioned how the control animals inoculated with +10 broth + chalk + tetanus toxin showed signs of tetanus and died more quickly than the animals inoculated with tetanus toxin in +5 broth. This fourth set of experiments corresponds to the former of the two controls and again demonstrates the greater tendency for tetanus toxin contained in +10 broth to which chalk had been added, to produce tetanic signs as compared with tetanus toxin contained in + 5 broth.

In spite of this, however, all the animals in the fourth set of experiments recovered and were alive and well months afterwards.

Of the fifth set of Experimental Animals inoculated with tetanus toxin contained in + 5 broth in which *b. Histolyticus* had been grown, all developed tetanus and died of generalised tetanus. The lowest/

lowest dose given here was slightly less than the reputed M.L.D. It was obvious from this that in spite of its very marked proteolytic powers, b. histolyticus was unable to destroy tetanus toxin, and in this respect differed markedly from the Reading bacillus.

III. THIRD SERIES OF EXPERIMENTS./

III. THIRD SERIES of EXPERIMENTS.

This series of experiments was conducted in order to determine the effect on tetanus toxin of b. sporogenes in doses higher than those employed in the first series. The same batch of toxin was used for the control as for the infected tubes, but whereas the latter were incubated anaerobically at 37° Cent. for nine days, the former was kept in the ice chest. The details are shown on the following tables. /i

1. Control animals inoculated with Tetanus Toxin only. dissolved in .85% sterile saline and kept in ice-chest for 9 days.

<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>		<u>Material.</u>	<u>Result.</u>
I. 0.0000014 grams		Tetanus Toxin dissolved in normal saline solution.	Died in 3 days of Generalised Tetanus.
II. 0.000038 grams			Found dying of Generalised Tetanus and killed at the end of 26 hours.

2. Animals inoculated with Tetanus Toxin contained in +10 broth infected with b. Sporogenes (Metchnikoff) incubated anaerobically for 9 days.

<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>		<u>Material.</u>	<u>Result.</u>
I.	0.000056 grams.	Filtrate from culture of b. Sporogenes grown in broth containing Tetanus Toxin.	Died in 7 days of tetanus.
II.	0.000099 grams	Ditto	Died in 4 days of tetanus.

The animal inoculated with the lowest dose in the control set died of generalised tetanus in three days. Both of the animals inoculated with Tetanus Toxin infected with b. sporogenes died of generalised tetanus, the smaller of the two doses given being considerably less than the highest dose of Tetanus Toxin rendered harmless by the Reading bacillus as shown in the second series of experiments.

iv. Fourth Series Of Experiments./

IV. FOURTH SERIES of EXPERIMENTS.

Having proved that the Reading bacillus was able in some way by its growth in tetanus toxin to modify that toxin so profoundly that even 148 times the M.L.D. was not followed by fatal results, I determined to find out if diphtheria toxin could be affected in a similar manner. This latter was chosen because of its high toxicity in small doses, and because of its ability to kill the animal within a few days.

Dr. A. T. MacConkey very kindly furnished me with a small supply of diphtheria toxin identifiable as No. 869, of which 1/100th of a c.c. was guaranteed to kill a guinea-pig of 250 grams weight within 4 to 5 days. This is equivalent to saying that a dose of .004 c.c. for every 100 grams of body weight was able to kill in 4 to 5 days. For ease of comparison I have again expressed the dosage in terms of so much for every 100 grams of body weight. Some of the diphtheria toxin was kept as a control. The rest was inoculated with the Reading bacillus, and all including the controls were incubated anaerobically for 10 days at 37° Cent. Of the inoculated toxin one part was filtered through a candle after incubation/

incubation, and the resulting filtrate inoculated in varying dose, while the remainder was inoculated without filtration. The details are set out in the following columns.

1. Control animals inoculated with pure diphtheria toxin incubated as stated above, and diluted with sterile 0.85⁰/o saline to make up the desired dosages.

Amount of Diphtheria
Toxin pro 100
grams of body
weight.

Material.

Result.

I.	0.46 c.c.	Diphtheria Toxin	Found dead in 18 hours.
II.	0.24 c.c.	Ditto	Found dead in 23 hours.
III.	0.1 c.c.	Ditto	Found dead in 25 hours.
IV.	0.01 c.c.	Ditto	Found dead in 48 hours.
V.	0.0042 c.c.	Ditto	Found dead in 3 days.

2. Animals inoculated with filtrate from Diphtheria

Toxin in which Reading bacillus had been grown anaerobically under the conditions stated above.

Amount of Diphtheria

Toxin pro 100
grams of body
weight.

Material.

Result.

I.	0.5 c.c.	Filtrate from culture of Reading bacillus grown in broth containing Diphtheria Toxin.	Death in 18 hours.
II.	0.24 c.c.	Ditto	No symptoms. Alive and well weeks afterwards.
III.	0.1 c.c.	Ditto	Ditto
IV.	0.01 c.c.	Ditto	Ditto

3. Animals inoculated with Diphtheria toxin containing

living Reading bacilli incubated as stated above.

Amount of Diphtheria

Toxin pro 100 grams
of body weight.

Material.

Result.

I.	0.49 c.c.	Living culture of Reading bacillus grown in broth containing Diph. Toxin.	Dead in 46 hours.
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Amount of Diphtheria
Toxin pro 100
grams of body
weight.

		<u>Material.</u>	<u>Result.</u>
II.	0.42 c.c.	Living culture of Reading bacillus grown in broth containing Diph. Toxin.	Dead in 3 days.
III.	0.24 c.c.	Ditto.	Alive and well weeks afterwards.
IV.	0.1 c.c.	Ditto	Ditto
V.	0.01 c.c.	Ditto	Ditto

(In each of these three sets of experiments the highest dose was injected intraperitoneally, the rest subcutaneously.)

The first set of experimental animals all died. That which had the smallest dose, very little more than the reputed M.L.D., died in three days.

Of those inoculated with the filtrate from the toxin in which R.B. had been grown, one, inoculated with 125 times the reputed M.L.D. died in 18 hours, while the others remained alive and well.

Of those inoculated with diphtheria toxin containing Reading bacilli which had been grown in it, two died. One which had received above 125 times the reputed M.L.D. died in 46 hours while the other, which/

which had 105 times the reputed M.L.D. lived for 3 days. These results compare quite favourably with those obtained with pure diphtheria toxin where 115 times the reputed M.L.D. was followed by a fatal result in 18 hours. The tendency observed here for the Reading bacillus to modify the diphtheria toxin is more marked, however, in doses somewhat smaller. Here we find that 60 times the reputed M.L.D. has no effect whatever on the inoculated animal. In other words the animal can withstand at least 60 times the M.L.D. of diphtheria toxin after the latter has been exposed to the action of the Reading bacillus. Probably the actual number of minimal lethal doses which a given animal would survive lay somewhere between 60 and 100 in this particular series.

V. FIFTH SERIES of EXPERIMENTS.

Having proved the ability of the Reading bacillus to modify the toxin of diphtheria, I was anxious to find out if in the same way it would modify the toxins of *Vibrio septique* and of *b. perfringens*. The former I had to abandon, as the doses of broth containing the toxic products were/

were too massive. In the case of the latter I was able to get some indication of the ability of the Reading bacillus to modify the toxin of *b. perfringens* which *b. histolyticus*, on the other hand, was unable to do. Cultures of *b. perfringens* were grown anaerobically at 37° Cent. for 13 days in broth containing minced bullock's heart. They were then filtered through candles and the filtrate divided into three portions. One was reserved for control purposes and was not inoculated. The second was infected with the Reading bacillus, while the third was sown with *b. histolyticus*. These three were then incubated anaerobically for 10 days, at 37° Cent. Various amounts of each were inoculated into animals, but only the highest of these doses proved fatal, indicating that rather a massive dose was necessary. The smaller doses, which were without effect, have accordingly been omitted. The highest dose in each case is given in the subjoined table.

	<u>Amount pro 100</u> <u>grams of</u> <u>body weight.</u>	<u>Material.</u>	<u>Result.</u>
I.	4.6 c.c.	Filtrate from broth culture of <i>b. per-</i> <i>fringens</i> .	Dead in 5 days.

	<u>Amount pro 100</u> <u>grams of</u> <u>body weight.</u>	<u>Material.</u>	<u>Result.</u>
II.	4.7 c.c.	Filtrate from culture of b. Histolyticus grown in b. perfringens filtrate.	Dead in 24 hours.
III.	4.7 c.c.	Filtrate from culture of Reading bacillus grown in b. perfringens filtrate.	No ill effect. Animal alive and well weeks afterwards.

(These were all injected intraperitoneally)

There were really two controls here, for the growth of b. histolyticus in the perfringens filtrate did not apparently render the latter non-toxic. This, again, is in line with the inability of b. histolyticus to destroy the toxins of tetanus. A somewhat similar dose of perfringens filtrate in which, however, Reading bacillus was grown, proved non-toxic, and the animal so inoculated remained alive and well. This last experiment, however, merely indicates that the perfringens toxin can be modified by the growth in it of Reading bacillus. It is, however, not very satisfactory, owing to the massive doses of perfringens filtrate necessary to produce a fatal result.

MANNER in which the BENEFICENT CHANGES are
wrought by the READING BACILLUS.

As a result of observations made on the character of gunshot wounds before ^{and} ~~the~~ after treatment with the Reading bacillus, and as a result of test tube experiments already mentioned, I was at first of opinion that the beneficial action of that organism was due entirely to its proteoclastic effect on the dead tissue present in the wound. I have already in this thesis made use of the analogy that the dead tissue plays the part of a base or depot from which pathogenic organisms are able to derive supplies which enable them to attack the living tissues yet undamaged.

Such an attack may be local, resulting in an extension of the necrotic process, or it may be directed against the tissues generally, consequent on the entry into the system of toxic substances elaborated by the organisms or split off in the course of their growth on the dead material of the wound.

This theory, however, did not quite explain all/

all the clinical phenomena observed in patients whose wounds had been treated with the Reading bacillus. I have already drawn attention to the rapidity with which the patient begins to improve after the wound has been sown with the specific anaerobe. The improvement may be said to commence almost at the end of 24 hours and generally at latest by the third day. It is a point which has impressed itself on all who have written about successful salt bag cases but one which has hitherto received no satisfactory explanation. It is one of the clinical characteristics of this form of wound-treatment. If the improvement depended entirely on the destruction and removal of the dead tissue by the Reading bacillus, one would scarcely expect beneficial results to follow until this process was completed or at least well advanced. At the time when constitutional improvement begins, however, proteolysis of the dead tissue is far from complete, and even when the packing is removed at the end of nine days there may still be a few emaciated sloughs left. While still convinced, therefore, that proteolysis of the dead material by the specific anaerobe is the chief factor making for

for recovery, it became necessary to take a wider view of the organism's range of activity. As the main point still awaiting adequate explanation, was the reason for the rapid improvement in the constitutional symptoms and as the latter were, in my opinion, probably caused by toxic substances constantly finding their way into the patient's system, two explanations in particular occurred to my mind.

In the first place the rapid improvement might be due to inhibition of the growth of pathogenic organisms either as a result of the formation by the Reading bacillus of some organic acid or acids or because of the starving out of these pathogenic members by the more vigorously growing anaerobe. To determine this point experiments were carried out in which the Reading bacillus was grown in culture tubes in symbiosis with various pathogenic organisms, the number of the latter being estimated at stated intervals. Details of these experiments have already been given in a previous part of this thesis and they seem to indicate that there is little, if any, inhibitory action on the part of the Reading bacillus. The latter could not be said to have outgrown the other with which it was living in symbiosis/

symbiosis nor was there any indication that the Reading bacillus produced any acid or other substance of an inhibitory nature. This experimental finding seemed further to be supported by the fact that the number of pathogenic organisms recoverable from pus in the wound at the time of removal of the packs appeared to be as great as before the treatment was commenced,

The same observation seemed further to indicate that no bacteriolytic enzyme^{is} produced by the Reading bacillus, although critical experiments have not yet been carried out to establish this point definitely. The second explanation that commended itself to my mind was this, namely that the Reading bacillus was able not only to attack and to break down dead tissues, but that it was also able to modify the toxic substances produced by the growth of pathogenic organisms present in the wound. In order to throw some light on this point, I resolved to expose a given toxin to the action of the Reading bacillus by growing the latter in broth containing some of that toxin. For this purpose I required a toxin of high potency, able to evoke definite clinical signs, capable of producing its effects in small doses, thus eliminating the danger of using massive quantities, and which at the same time/

time could be accurately measured and inoculated in graduated doses for purposes of comparison. Tetanus toxin seemed to fulfil these conditions, and this accordingly I chose. The experiments bearing on this question have already been described, and seem to furnish clear and conclusive evidence that of all the organisms investigated, the Reading bacillus alone was capable of destroying the toxin of tetanus and of protecting the animal against nearly 150 times the M.L.D. The only organism comparable in this respect to the Reading bacillus was *b. Sporogenes* (Metchnikoff) but the effect of the latter was only partial. It is interesting to note in this connection that of all the anaerobes with which I have had the opportunity of comparing the Reading bacillus, *b. Sporogenes* is the most closely related. Probably these two organisms are simply two slightly different strains of the same species. Henry,³⁵⁾ dealing with the cultural reactions of anaerobes isolated from wounds, seems to have formed a similar opinion when he says that not unlikely one is dealing with a whole group of bacilli rather than with a single individual. The identity of the Reading bacillus with *b. Sporogenes* (Metchnikoff) I am now investigating, and I am not therefore in/

in a position to make any definite statement at this juncture.

To return, however, to the subject of this thesis, we have seen that the Reading bacillus was able to destroy the toxin of tetanus. Having established this fact, I resolved to find out if this were merely a selective action on tetanus toxin only, or if it would attack, in like manner, diphtheria toxin for instance. Experiments related above were carried out with this end in view, and again there was very definite evidence of de-toxication by the Reading bacillus. Further experiments also furnished indications of a similar power on the part of the specific anaerobe to modify the toxin present in filtrates obtained from cultures of *b. perfringens*. Here, however, practical difficulties stood in the way of obtaining clear and defined results, as the volume of filtrate necessary was too massive in relation to the size of the animal used for inoculation.

Enough, however, had been done to show that the toxins both of tetanus and diphtheria were in some way rendered harmless by the growth in them of the Reading bacillus, while there was also an indication/

indication that the toxic substances produced by *b. perfringens* were in like manner modified. While this effect of the Reading bacillus has been proved only in respect of the above three groups of toxins, it seems legitimate to assume that toxic substances produced by other pathogenic organisms will be acted upon in a similar manner and so robbed of their toxicity.

This conception, based partly on experimental grounds, seems to offer an explanation of the rapid improvement which occurs in the patient's general condition. It suggests that the constitutional recovery is due largely or in considerable measure to the vital activity of the Reading bacillus on the toxic substances produced or set free by pathogenic organisms, while the actual cleansing of the wound is going on as a result of proteolysis of the necrotic or dying tissue by the same specific anaerobe.

This at all events is a reasoned attempt, based partly on clinical and partly on experimental grounds, to explain the method of working of an organism whose power to cleanse wounds and hasten convalescence is an undoubted clinical fact. Most bacteria, however, are known to/

to form enzymes of one kind or another, and as the outstanding function of the Reading bacillus was to break down protein, to judge from its clinical results and cultural reactions, it was probable that its proteolytic activity depended on an enzyme or enzymes, probably of the nature of a protease. Assuming that this was so, the possibility of isolating the enzyme or enzymes from the causal organism presented itself.

In view of the fact that an apparently Ecto-cellular enzyme may be really an Endocellular one liberated on the death of some of the bacilli containing it, and that under cultural conditions it would be difficult to prove the presence of a purely ectocellular product, my intention was merely to find out if the enzyme could be found in appreciable amount in an extracellular form, leaving aside the question of its being an excretion product. To this end cultures of the Reading bacillus were put up in broth with a +5 reaction. After incubation for ten days anaerobically at 37° Cent., these were filtered through a porcelain candle. One c.c. of the clear filtrate was then superimposed on the surface of a column of sterile gelatin which was left at room temperature. This slowly underwent liquefaction/

liquefaction beginning at the top and spreading downwards till the whole became fluid. The action was progressive, but solution did not occur with anything like the same rapidity as does a gelatine tube infected with the living bacillus. To another another tube containing egg albumen coagulated by heat, were added a few c.cs. of the filtrate and the tube was then kept at 37° Cent. The coagulum gradually broke up, the fragments became more and more translucent, and in a few days the whole had disappeared. Proteolysis was complete. These two simple experiments appeared to show that the Reading bacillus was able to elaborate an enzyme which could easily be separated from the living organism, and which was still capable of definite proteoclastic action. Its behaviour in relation to gelatin and to coagulated egg albumen suggested a similarity to the animal ferment trypsin, and accordingly one or two simple experiments were made by way of comparison. For the same purpose also a vegetable enzyme, papain was chosen. The results obtained from the latter, however, were only partial, probably because an optimum temperature was not observed, and they have accordingly been omitted.

Into/

Into each of a series of tubes containing sterile distilled water maintained at boiling point, was dropped 1 c.c. of egg albumen. When cool, the water was pipetted off from the coagulum. To each of a certain number of these tubes were added 5 c.c. of different dilutions of trypsin, ranging from 1 in 50 to 1 in 10,000 dissolved in 1% Na_2CO_3 . To another were added 10 c.c. of filtrate from a nine day old culture of the Reading bacillus in 5 broth. These tubes were then placed in the 37° incubator and observed from time to time. To yet another tube were added 5 c.c. of sterile .85% saline solution, which was then inoculated with the Reading bacillus, and thereafter incubated anaerobically at 37° Cent. It was found that the coagulum of egg albumen had disappeared in 4½ days from the tube containing the filtrate of Reading bacillus. The strength of trypsin solution which effected the same change in the same time lay between 1 in 1500 and 1 in 2000. The coagulum in saline containing living Reading bacillus had disappeared, however, within 60 hours. Other two tubes, containing the same volume of minced bullock's heart were next observed. To one of them 1 c.c. of a 1 in 50 solution/

solution of trypsin in 1⁰/o Na₂CO₃ was added and to the other 1 c.c. of an old broth culture of the Reading bacillus. These tubes were then incubated aerobically at 37⁰ Cent. Within 24 hours the volume of meat in the trypsin-containing tube had begun to diminish, while as yet there was no alteration in the Reading bacillus tube. Although the trypsin had the start of the organism, at the end of 4 days the meat in each tube stood at the same level. The volume in each case had diminished by 2 centimetres. Six days later the tube infected with Reading bacillus was leading. The volume of meat in the latter tube had diminished to the extent of 2.6 centimeters while that in the trypsin tube had only decreased by 2.3. In both tubes however it was obvious that the reaction had commenced to slow down. Presumably in both cases as the products of hydrolysis accumulated, a point of equilibrium was being reached, but apparently more rapidly in the case of the trypsin. A solution in sterile .85⁰/o saline of 1⁰/o soluble casein, was inoculated with the Reading bacillus and incubated anaerobically for 10 days at 37⁰ Cent. At the end of this period the addition of 1⁰/o acetic acid was unable to bring down/

down any precipitate, whereas an equal amount of the same acid added to some of the casein solution to which the organism had not been added brought down a heavy precipitate of casein.

Finally, chemical tests applied to these fluids containing proteins which had been acted upon by the Reading bacillus yielded reactions very similar to those obtained from proteins which had undergone tryptic digestion. In both cases there was evidence of the proteolytic action going on to the amino-acid stage.

In view of the fact ^{then} that the Reading bacillus appeared able to produce a fairly powerful enzyme closely resembling trypsin in its action, the question next arose as to whether the power of the specific anaerobe to detoxicate tetanus toxin was the result of a specific enzyme or whether it was due to its general proteoclastic activity. To answer this question I decided to expose tetanus toxin to the action of a known proteolytic enzyme and because of the resemblance of the enzymic properties of the Reading bacillus to those of trypsin, I chose the latter for the purposes of experiment. Two solutions of powdered trypsin were made. One of the solvents was sterile/

sterile distilled water containing 0.85% Na Cl, the other, sterile distilled water containing 1% hydrated Na_2CO_3 . The strength of the trypsin solutions was 1 in 50. A weighed quantity of the same sample of dessicated tetanus toxin as had been used for previous experiments was dissolved in a known amount of sterile .85% saline, and measured quantities of this were added to the solutions of enzyme so that the amount of tetanus toxin contained in each c.c. of the enzyme solutions was known. The tubes were then incubated for nine days at 37° Cent. This was done anaerobically and the tubes kept in the dark so that the tetanus toxin might not deteriorate by being exposed to air or light.

The results of these experiments are exposed in the subjoined table.

1. Control animals inoculated with Tetanus Toxin alone.

<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>	<u>Material.</u>	<u>Result.</u>
0.0000014 grams.	Tetanus toxin in solution kept for 11 days in ice chest.	Signs of tetanus in 24 hours. Died of generalised tetanus in 3 days.

2. Animals inoculated with Tetanus Toxin exposed
to the action of Trypsin.

<u>Amount of Tetanus Toxin pro 100 grams of body weight.</u>		<u>Material.</u>	<u>Result.</u>
I.	0.000001 gram	Tetanus Toxin exposed for 9 days to action of 1 in 50 Tryp- sin in 10/o Na ₂ CO ₃ .	No sign of Tetanus. Alive and well weeks afterwards.
II.	0.000097 grams	Ditto	Ditto

It is at once obvious from these experiments that trypsin likewise possesses the power of rendering the toxin of tetanus harmless to guinea-pigs, and the end result appears to be similar to that obtained from the growth of the Reading bacillus in a medium containing the above mentioned toxin. It does not appear therefore that the Reading bacillus is endowed with a specific enzyme not possessed by trypsin. On the other hand, it seems extraordinary that an organism like *b. histolyticus* possessed of very strong proteolytic power should nevertheless be unable to effect the substrate of tetanus toxin. It would appear that while most bacteria possess the/

the power of attacking protein, only a few possess the power of forming proteases in any appreciable amount, and probably still fewer possess the power of hydrolysing proteins in such a way that the destruction products are themselves non-toxic. That the Reading bacillus appears to belong to this select group, seems proved by the clinical and experimental observations already recorded. Neither in patients whose wounds have been liberally sown with this organism, nor yet in experimental animals has there ever been the slightest evidence that the destruction products of its prote^eolytic action have any toxic effect whatever, or that the organism has any power over living tissue. Not only so, but it appears both from clinical evidence, if my interpretation of that is correct, and from experimental work to possess the added power of breaking down bodies of a toxic nature. The investigation has therefore resolved itself now into a study of enzymes and enzyme action and as such has become involved in problems concerned with the chemistry of colloids, problems which probably only the bio-chemist is capable of solving.

The gun-shot wound is a solution of continuity of/

of the body, produced by violence, and characterised by a greater or less amount of dead or dying tissue in juxtaposition to the living and less damaged structures. This dead tissue is more or less heavily infected with organisms, most of them pathogenic and many highly virulent. Trouble, local and constitutional, arises from the interaction of pathogenic organisms with the necrotic tissue. As a result, bacterial ferments possessing toxic properties are probably produced. To these must be added leucocytic and other tissue ferments liberated in the course of the morbid process. As a result of the multiple hydrolytic action taking place, degradation products, many probably of a toxic character result. Some of these act injuriously on partially damaged or undamaged tissues adjacent, so that the process of necrosis goes slowly on involving more and more of the living structures. Others probably enter the patient's system, giving rise to toxic symptoms, of which evidence is afforded by the temperature chart, the pulse and other constitutional disturbance. In order to counteract the destructive processes, artificial methods of attack have been employed, and these may be divided into two main categories/

categories. The first includes all those methods which aim at setting a limit to further breaking down of the tissues. To this group belong nearly all the usual methods of wound treatment. It includes, for instance, practically all antiseptics with perhaps the notable exceptions of Eusol and Dakin's solution, as I have indicated in an earlier part of this thesis. With the exception of the two latter, the rest are directed against one factor only in the morbid process. They aim at destroying the pathogenic organisms or at inhibiting their growth, leaving nature to cast off slowly the dead tissue. Apart from the fact that this method overlooks the all-significant presence in the wound of necrotic tissue, such methods are probably at best to be regarded as crude. Not only can they not be relied upon to render the wound sterile or to prevent the continued absorption by the patient of toxic products, but they may actually cause fresh tissue necrosis. At best they may be regarded as a means of keeping within reasonable limits, bacterial activity what time nature is endeavouring to cast off the dead sloughs which the antiseptics are as a rule powerless to touch. The healing of a gun-shot wound under such circumstances must/

must be at best a tardy process liable at any time to be interrupted by renewed organismal activity, and for these reasons requiring, as I have animadverted on in the clinical section of this thesis the frequent assistance of minor operative measures. During all this time, the patient is probably absorbing more or less of the toxic products which in turn may initiate morbid changes in some other of his tissues. Indeed, the absorption of such toxic substances over a protracted period may have the same end-result as an initial overwhelming infection. In view of all these facts one is almost tempted to say that an antiseptic is really a contradiction in terms.

In sketching the outlines of wound treatment during the present war, I have shown that the comparative failure of many of the antiseptics has led to the substitution for them of a method of treatment by excision. This method falls also within the first category, inasmuch as it endeavours to put an end once and for all to further breaking down of the tissues by the rapid removal of the organisms together with their breeding ground. In other words, the excision method takes into account not only the organisms but the dead tissue as well whose importance is/

is so often lost sight of by those who advocate anti-septics. Most of the methods included in the first category possess serious limitations to which I have referred frequently in these pages.

In the second category, which ought to include all artificial methods directed towards acceleration of the process of proteolysis taking place in the wound, I was inclined at first to think that there was only one, viz. the biological treatment of the wound by means of the Reading bacillus. I must modify this, however, and say that the biological method is the only one in this category so far whose rationale depends on an organic catalyst produced by a living organism. I make this modification because there are indications, as I shall mention presently, that preparations of the Eusol type may act to a certain extent in a similar direction.

The Reading bacillus not only possesses the power of accelerating proteolysis and removal of dead tissues from a wound, but it seems endowed with the further property of being able to render non-toxic the degradation products of pathogenic organisms. It is able not merely to bring about a dissolution of the breeding ground of such bacteria/

bacteria, but it is able speedily to put an end to the continued absorption of toxic products by the patient which is one of the drawbacks of the ordinary antiseptic method. Over and above all this, and it is a point of considerable importance, the Reading bacillus confines its activities to the dead tissue and does not extend its hydrolytic action to the living structures. It has further very decided advantages to which I shall refer presently.

It is probable that the application to the wound of a solution of such a ferment as trypsin might act in a somewhat similar manner. Indeed, I have already quoted an instance of its use for this purpose by a German field medical officer; while, as an empirical attempt in the same direction may be instances³⁴⁾ the immemorial use of the leaves of *Pinguicula* (butterwort) by the shepherds in the Alps as a cure for ulcers on the udders of cows. The therapeutic value of these leaves appears to depend on a vegetable trypsin by means of which the plant is able to proteolyse the bodies of insects caught on the leaves. The Reading bacillus, however, possesses certain marked advantages over the use of such a ferment as trypsin. The latter, to be of any/

any value, must be kept constantly renewed, since much of it will speedily be carried away in wound discharges, whereas the Reading bacillus, once implanted, and given anaerobic conditions for growth, will remain active as long as there is necrotic material to hydrolyse. The anaerobe is a permanent manufactory of a proteoclastic enzyme whose initial velocity will be more or less maintained throughout, owing to constant removal by the wound discharges of the products of ^{its} ~~the~~ hydrolysis. Probably to this category also, belong such substances as Eusol and Dakin's solution, in virtue of their strong proteolytic properties on which, as I have already said, Dakin himself lays very considerable stress.

Morgan, Saner and Schlesinger, in a recent ³²⁾ paper take the very decided view that Eusol as an antiseptic is quite unimportant, but that its great and undoubted value lies in its power of destroying dead tissue, so depriving the infecting organisms of their pabulum.

This is due in their opinion to hydrolysis of the protein, and as it is a bulk chemical action, the amount to be used must bear a direct ratio to the amount of protein to be destroyed. Such a view of/

of its action, if substantiated, brings Eusol into the same category as the Reading bacillus. Like the latter organism, its chief function is to accelerate proteolysis. It would seem to play the part of an inorganic catalyst, and to this extent differs from the Reading bacillus. The latter, indeed, possesses an important advantage in wounds over Eusol inasmuch as the catalytic agent of the bacillus is an enzyme which is constantly being manufactured in the wound, and whose action is continuous and not subject to the limitations of a substance like Eusol which is dependent on bulk chemical action. The claim of Eusol to be regarded not merely as an antiseptic is further strengthened by the extraordinary results obtained by Professors Lorrain-Smith, Ritchie and Dr. Rettie³⁵⁾ in certain cases by the intra-venous injection of Eusol. Their results suggest that the antiseptic property of Eusol is relatively of secondary importance compared with other properties which it possesses. The authors seem to be of opinion that the majority of so-called septicaemias are really of the nature of toxæmias, a view which, as I have indicated in an earlier part of this thesis, I felt constrained to take in trying to understand the rationale of the Reading bacillus/

bacillus treatment. In such cases of toxaemia the benefit of Eusol introduced directly into the circulating blood may be due, according to its authors, directly to a destruction of the toxin or indirectly to the stimulation of a protective reaction. Much the same thing appears to take place in the wound treated with the Reading bacillus. Apart from the latter's proteolytic action on dead tissue requiring speedy removal, there is also apparently a power of preventing the absorption by the patient of the destruction products arising from the activity of pathogenic organisms present. Experimental proof has been furnished in an earlier part of this thesis of the power of the Reading bacillus to rob certain toxic substances of their power to kill. This property is probably vested in the enzyme or enzymes produced by the bacillus and as these are of a colloid nature, we have some indication of the probable mechanism whereby a stop is put to the constant absorption by the patient of toxic bodies.

In this connection Dean and Adamson^{36/} have demonstrated a somewhat similar effect by exposing the toxin of *b. dysenteriae* (Shiga) to the action of a weak solution of Eusol. This experiment, which further supports the/

the contention that Eusol possesses an important action other than a merely antiseptic one, seems to fall into line with my own experiments on the toxins of tetanus, etc. exposed to the action of the Reading bacillus. Whether or not the toxin of tetanus, rendered innocuous in the way I have described, is still capable of inducing immunity, I have so far not yet determined, although I am conducting experiments in this direction. In view, however, of Dean's results, it is obvious that such experiments may lead to very important developments in the production of immunity.

It would appear that the biological method of treatment and that by means of Eusol, begun independently, and followed along different lines, will ultimately converge and find an explanation in a common basis.

In the case of the Reading bacillus, I feel that the explanation of its action is probably bound up with the properties of its enzyme or enzymes, and this carries the problem into the domain of colloid chemistry, where, so far as this thesis is concerned, it must be left for the present. Sufficient, however, has been said to indicate the multitude of/
of/

of problems that arise in connection with this conception of wound treatment and the far-reaching possibilities to which it may give rise. Certainly it seems to suggest that the cruder methods now in use for combating wound infection will ultimately give place to methods of a more scientific character based on a knowledge of the actual biochemical phenomena involved in the morbid processes occurring as a result of wound infection.

SUMMARY AND CONCLUSIONS.

1. Three main methods of wound treatment have characterised surgical procedure during the present war, viz., the antiseptic, the physiological and the surgical i.e. by Excision of the wound in toto. The latter has come into being partly owing to disappointing results obtained by the two first-named methods and partly because, by its adoption, the convalescence of the wounded is hastened.
2. From the physiological method by the use of hypertonic saline solutions, has evolved, as an offshoot, the salt pack treatment of wounds and extraordinarily good results are claimed for it by its advocates.
3. Hitherto the beneficial results following salt bag treatment have been loosely attributed to the presence of the salt acting more or less on the physiological lines suggested and described by Wright. From a study of the various published articles which discuss this method of treatment, it is evident that Wright's theories are inadequate as an explanation.

4. This thesis offers an entirely new explanation of the phenomena observed and will, I venture to hope, supply the key to much that has hitherto been obscure.
5. The work which I carried out and which furnished the clue to this explanation, is based, in the first instance, on a clinical observation made by one of my colleagues, viz., that all the wounds which did well under salt-pack treatment were characterised by a peculiar offensive odour which was absent on the other hand from wounds which did not so improve.
6. This clinical observation suggested to my mind the possibility that a certain organism might be present in the wounds characterised by this odour but absent from those which were not so distinguished.
7. Acting on this assumption I discovered that a certain bacillus was apparently always present in wounds emitting this peculiar odour while it was absent or could not be recovered from wounds which did not smell.
8. This bacillus is a spore-bearing anaerobe of a saprophytic nature and belongs to the proteolytic group/

group of organisms - the group which includes bacillus tetani and bacillus oedematis maligni (Koch).

9. Unlike these latter it does not appear to be pathogenic for animals, and what is of much more importance, is non-pathogenic for man when introduced in living culture into open wounds.

Further, unlike the pathogenic members of this group it does not appear to set free, in the course of its action on dead tissue, toxic products injurious to the patient.

10. I have called the organism temporarily the "Reading bacillus" inasmuch as it may previously have been described and may already possess a name. Owing to the somewhat vague descriptions which are generally given of anaerobes, I have taken no definite steps to identify it with any other known strain. While, for example, it seems to resemble in most respects, the b. oedematis maligni of Koch yet it does not appear to coincide exactly with the latter and moreover such a name possesses a sinister connotation which it is inadvisable to associate with the Reading bacillus.

11. I have endeavoured rather to furnish such complete details/

details of its morphological and cultural characters as will enable it to be recognised by other bacteriologists. In addition, I have given particulars of experimental work performed on animals with a view to establishing its Claim to non-pathogenicity and in order to learn something of its probable mode of action. In connection with the latter, certain other experimental work is described in detail.

12. Descriptions are also given of a new method of isolating anaerobes from mixed cultures and of an improved form of anaerobic plate which the above investigation has led me to devise.
13. The Reading bacillus is probably present along with other organisms in the majority of infected wounds but conditions favourable to its growth and development are not furnished by the various methods of wound treatment in use with the exception of that by means of salt packs.
14. I have endeavoured to show, however, that the salt, as such, has no particular virtue in promoting growth of this organism. On the contrary, experiments described in this thesis tend to show that growth is actually retarded by the presence of high/

high concentrations of salt. It is obvious, therefore, that there is no need to call in Wright's theories to furnish an explanation of the success attending the salt bag treatment of wounds.

The real reason appears to depend on the fact that by this method, the wound is rendered more or less anaerobic so permitting the development of the Reading bacillus. The salt can be omitted with impunity so long as an effective packing is substituted which will provide the necessary anaerobic conditions.

15. I have laid special stress on the necessity of recognising that one of the chief factors which keep a wound septic, is the presence of devitalised tissue in that wound and I have ventured to suggest that the surgical definition of a wound be amended so as to take cognisance of this factor.

Failure to estimate sufficiently the importance of this dead tissue probably explains the partial want of success which has characterised the antiseptic and the physiological methods.

16. The surgical or excision treatment of wounds, although aimed at the early and rapid removal of pathogenic organisms, actually does remove the bulk of the dead tissue as well, and, in so far as/

as it does this, it differs radically both from the antiseptic and from the physiological methods. For the same reason also it is superior to them, and the results, following treatment by excision, are better.

17. Such a method, however, is not always anatomically possible, and while successfully removing macroscopically dead tissue, it may fail to remove the necrotic material less obvious to the naked eye. Moreover in doing so, it inflicts a fresh trauma and leaves behind a zone of death liable to re-infection.

18. Instead of these, I now venture to advocate what I have called the "biological" method, a method which has now been in routine use for some time in Reading. It is not to be confused with the salt bag method of treatment, although the latter depends for its success on the former. The biological or bacteriological method consists in the sowing of the wound with living cultures of the Reading bacillus and the subsequent dressing of the wound in such a way that more or less anaerobic conditions are brought about.

The/

19. The employment of the biological method does not mean that no surgical interference is necessary. Here as in every other method it is essential that the wound be thoroughly laid open in the first instance, exposing every pocket and sinus so that the organism and the packing may be brought into direct contact with the wound surfaces.
20. The advantages of the method include simplicity of application, the avoidance of daily dressing and daily disturbance of the wound, the rapidity with which a sloughy wound becomes a healthy granulating surface, the absence of secondary haemorrhage together with the remarkable and speedy improvement which takes place in the general condition of the patient.
- All this means considerable curtailment of the time generally spent by a wounded man in hospital.
21. A series of 20 clinical cases of gun-shot wounds has been chosen for the purpose of illustrating various points raised in this thesis and in a commentary appended to each, their significance is discussed at some length.
22. Treatment with the Reading bacillus appears to be followed by improvement in two directions.
- All/

All dead material is rapidly removed from the wound and with it the pabulum for the pathogenic organisms. Prior to this, however, there is a more rapid improvement in the patient's general condition, the reason for which has hitherto not been fully understood.

23. The local improvement is shown to depend on the proteolytic activity of the Reading bacillus, while experimental data seem to prove that the constitutional recovery is probably due to interference with the absorption of toxins by the patient.
24. The improvement in both cases appears to be due to the enzyme action of the Reading bacillus, whose ability to destroy toxic bodies in general, is suggested by experiments on tetanus and other toxins, whereby many times the minimal lethal dose of these toxins can be tolerated with impunity.
25. The application to sloughing wounds of a living organism apparently capable of effecting by means of an organic catalyst, the hydrolysis not only of dead tissue but also of toxic substances produced by pathogenic organisms, without itself at the same time giving rise to degradation products/

products of a harmful character is probably an entirely new method of treatment and differs in this respect from all other methods of treatment in vogue. It is one pregnant with possibilities for the future, suggesting as it does, new lines of research which may throw light on questions still obscure and may lead to further important therapeutic developments.

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